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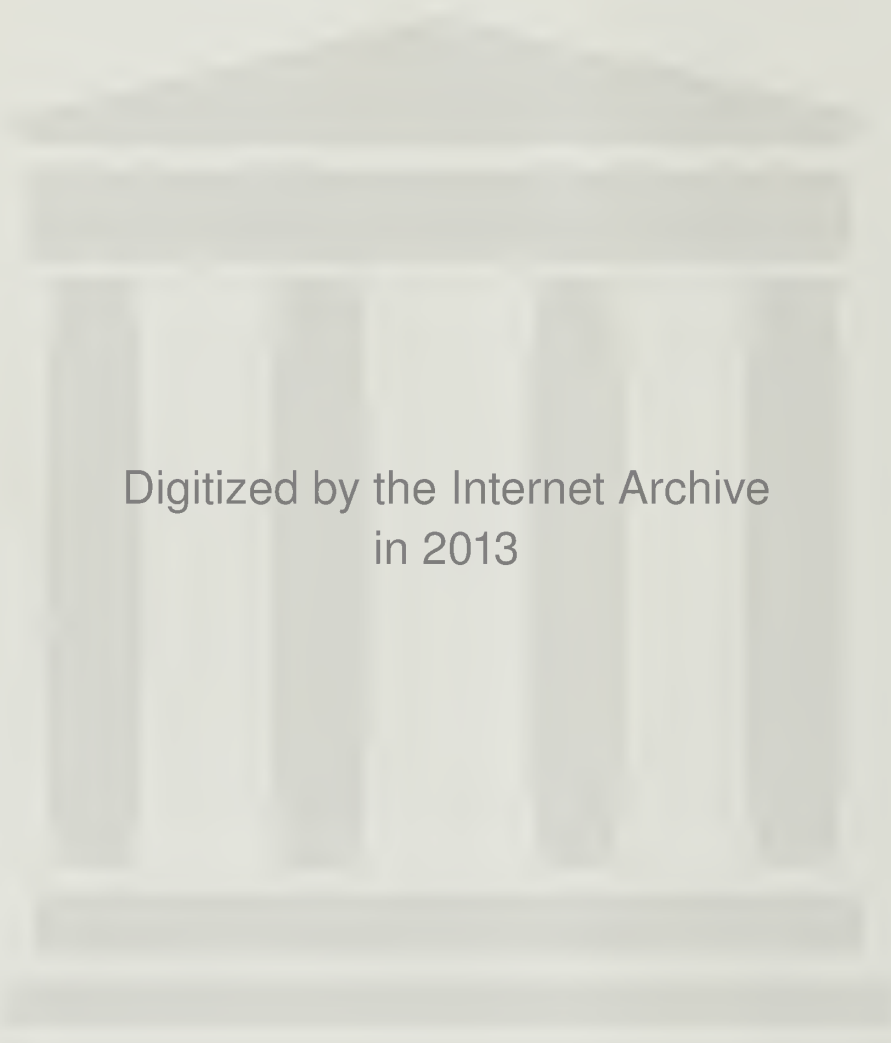
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BIOLOGICAL REMOVAL OF COLLOIDAL WASTE IN ACTIVATED SLUDGE PROCESS

By

SHANKHA KUMAR BANERJI

Supported by

DIVISION OF WATER SUPPLY AND POLLUTION CONTROL

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Shankha Kumar Banerji, Ph.D.

Department of Civil Engineering

University of Illinois, 1965

Although colloids represent a significant fraction of the organic pollutants in domestic and many industrial wastes, the literature contains scant information on the removal characteristics of colloidal wastes in aerobic or anaerobic biological treatment.

The kinetics and mechanism of the removal of a colloid, potato starch, was determined in a batch activated sludge system under different operating conditions. The variations of the operating conditions chosen were food-to-microorganism ratio (F/M), temperature, acclimation, and oxygen deficiency. The pH was maintained constant at 7.2 in all the experiments and there was no deficiency of inorganic nutrients in any system.

The results showed that under acclimated systems the total-Chemical Oxygen Demand (COD) removal rate was linear under almost all operating conditions. The removal rate constant of total-COD was directly proportional to the initial mixed liquor suspended solids (MLSS) up to a limiting value depending on the temperature.

The starch-COD removal, representing the degradation of starch molecule by exoenzymes, was logarithmic for initial periods in all cases. There was some divergence from this logarithmic removal characteristic of starch-COD at later stages. This was attributed to increased microbial population and changed substrate structure by the enzyme action. The rate

of starch-COD removal at different temperatures was proportional to the inverse of F/M ratio up to a limiting value of F/M. This was attributed to the enzyme saturation effect.

The degradation products of starch liberated in the medium were small-sized carbohydrate molecules which were utilized by the cell as reflected by the total-COD removal rate after all the starch-COD was eliminated.

The effect of temperature (5°, 20° and 30°C) on rate of removal of starch followed Van't Hoff-Arrhenius relationship.

In oxygen deficient systems the removal rate of starch-COD was similar to that in aerobic conditions, but the removal of total-COD and total-carbohydrate was severely affected. There was practically no synthesis of sludge, although the initial adsorption of starch was unaffected.

In the non-acclimated systems the rates of removal of starch were much lower than in the acclimated systems and, in some cases, there was a significant lag period before the removal kinetics were established. With glucose, maltose and Metrecal sludges, the enzymes responsible for starch degradation were constitutive, but in bovine serum albumin sludges the enzymes (amylases) were adaptive.

Degradation of starch by acclimated sludge filtrate (cell-free) after some minutes of contact was only about 2 percent of the starch degradation of whole cells. This low cell-free starch degradation activity of the sludge filtrate was found to be due to the location of the starch degrading enzymes on the cell-wall rather than in the medium. Isolated cell-wall preparations had higher starch degrading activity when compared to whole cells.

Adsorption of starch by sludge was prevalent in all systems

tested. The amount of starch adsorbed varied with acclimation, MLSS and in some cases temperature. There was no correlation between the amount adsorbed and the starch concentration in the medium. However, generally the adsorption was higher with high sludge concentration. Acclimation was very important for starch adsorption and most non-acclimated systems adsorbed starch much less than the corresponding acclimated system. Thus, the adsorption of starch on cells is more than just a physico-chemical process.

The mechanism of removal of starch in activated sludge systems is the initial adsorption on the sludge cell surface followed by enzymatic degradation by the cell-wall located enzymes. The degradation product of starch in carbohydrate form would be liberated in the medium and assimilated by the cells.

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1. INTRODUCTION

At the present time, by far the most important method of organic waste treatment is biological treatment. Since its inception in the late nineteenth century and early twentieth century, biological waste treatment has made tremendous strides. Various industrial wastes which were considered to be toxic to the microorganisms only 10 to 15 years ago have been successfully treated by the use of the proper type of microflora. Although there are still some unanswered questions relating to the treatment process, significant advances have been made towards explaining the biochemical and physical nature of the process for simple substrates, especially in the activated sludge process. The contributions from the fields of microbiology, biochemistry, and physiology have proved invaluable in the understanding of the complex activities of microorganisms in the waste treatment works. This clearer understanding of the biological treatment process (especially with soluble wastes) provides a sounder basis for the design of the treatment facilities and has reduced the pollutional loads on streams by thousands of pounds.

The understanding of the removal of colloidal and particulate wastes in biological waste treatment is still very scanty (1). The colloidal fraction of a waste may vary according to the character and origin of the waste. In domestic sewage, Balmat (2) estimated that colloids represent as much as 52 percent of the biological oxygen demand (BOD) and 54 percent by weight of the suspended solids of the sewage. Some industrial wastes may have a higher content of colloidal organic load. For example, wastes from the textile desizing process contain large quantities of starch (3); waste from paper and pulp factories and food processing industries have a high

colloidal content also. Casein in dairy wastes, protein water in wastes from the potato starch manufacture, and blood in the slaughter house wastes are other examples of colloidal macromolecules in industrial waste. Despite the fact that various chemical precipitation methods have been developed for the treatment of colloidal wastes, the ultimate disposal of the sludge produced is often accomplished by biological methods. Existing methods of biological waste treatment which successfully remove colloidal matter either alter the hydration and electric forces, resulting in agglomeration and settlement, or produce exoenzymatic degradation of the colloidal molecules to sizes small enough to permit utilization by the microorganisms.

There is a dearth of literature on the treatment of colloidal wastes by biological means, although it is established that colloids in waste constitute a significant part. One reason for the lack of research on this aspect of the problem could be the difficulties of analytical procedures for determining colloids in the presence of microorganisms, which themselves are of colloidal size.

The present work was undertaken to determine the basic physico-chemical and biological factors responsible for the removal of a purely colloidal substrate in an activated sludge system.

2. LITERATURE REVIEW

The colloidal fraction of sewage has attracted the interest of many investigators. The early workers were mainly interested in separating and estimating the amount of colloids present in the sewage (4)(5). They assumed an arbitrary distinction between the suspended particulate and colloidal matter, and made no attempts to critically study the method of differentiation adopted in their investigations. It was Mills (6) in 1932, who first systematically investigated the sewage colloids and classified them according to their particle size. This work was primarily concerned with the occurrence and stability of the colloids as affected by factors like pH, ionic strength, and other cations.

Balmat (2) in 1955, using improved techniques, classified sewage colloids on the basis of particle size. He was mainly interested in separating various particulate and colloidal fractions, chemically characterizing them and studying the BOD removal rate of these fractions. He concluded that colloids (one micron to one millimicron size) and supracolloids (nonsettleable solids greater than one micron size), represented as much as 52 percent of the total BOD and 54 percent by weight of the suspended solids in the sewage. Heukelekian and Balmat (7) reported the mineral and organic composition of the different fractions of sewage, indicating that colloids have high calcium and phosphorus, and supracolloids have high phosphorus, silicon, and calcium contents. They also attempted to separate the fractions by solvent extraction into total grease (soluble in petroleum ether), alcohol-soluble (exclusive of grease and containing amino acids) and alcohol-insoluble (containing polysaccharides) fractions. Balmat (8), using a Warburg respirometer, reported the BOD removal kinetics

of the colloidal fraction of the sewage. The rate constant, k_1 (common logarithm), for supracolloids, was found to be 0.09 and that of the colloids was 0.22. He indicated that comminution of some wastes to one micron size or smaller would greatly increase their rate of biochemical oxidation. No report has been published characterizing the components of sewage which are soluble but of colloidal size. This category includes various polysaccharides like starch, dextrans, glycogen, etc., some proteins like albumins, hemoglobin, etc., and other macromolecules soluble in water.

The first study involving purely colloidal substrates, i.e., starch and dextrin, in an activated sludge system in the laboratory was reported in 1947 by Placak and Ruchhoft (9). It was observed that very high initial removals, 30 to 80 percent, were obtained when activated sludge systems were fed these substrates, followed by a continuous removal over the 22- to 24-hour aeration period, so that the total removal was over 90 percent. The high initial removal was attributed to adsorption of the substrate by the sludge. However, they did not pursue this finding under different conditions and neither did they try to correlate the initial uptake with the overall removal kinetics of the substrate. Thus, their results were of a more qualitative nature than quantitative. Gaudy (10), using a glucose acclimated sludge, obtained a high initial removal of glycogen substrate. Although the increased sludge did indicated adsorption, he found on analysis that the sludge did not have an equivalent high carbohydrate content. He concluded that the adsorbed carbohydrate was probably used immediately for synthesis of protein in the cell. With soluble starch, he did not obtain any initial removal and concluded that adsorption was not prevalent in this case. This discrepancy between the behavior of glycogen and starch was attributed to the fact that the glycogen

substrate was not completely soluble and had the appearance of a colloidal suspension, whereas the starch was completely soluble. This explanation does not seem reasonable, since even soluble macromolecules can be adsorbed on surfaces, and the adsorption of soluble proteins on soils is an excellent example of this (11). Gaudy (10) was investigating the effect of a qualitative shock load of starch and dextrans to glucose acclimated cells, and so he did not study the kinetics of the removal of these substrates in greater detail, nor did he further investigate the high initial adsorption phenomenon observed with glycogen.

In the industrial waste field, there are numerous references to the biological treatment of colloidal waste; for example, dairy wastes (12) (13), textile wastes (14)(15), potato starch industry waste (16)(17), and citrus waste (18). No unified approach to the study of the decomposition rate or mechanism for the colloidal components has been reported. Usually the colloid, be it protein, carbohydrate, or lipid, is present together with various other organic matter, some smaller (soluble) and some larger (particulate) matter. Therefore, any conclusions drawn from studies on these mixed wastes would not be entirely representative of removal of the colloids.

Chemical treatment (19)(20) has also been used for the removal of colloidal components of industrial wastes as mentioned earlier. With chemical treatment, precipitated colloids are difficult to dewater, sometimes become a waste disposal problem, and often require anaerobic digestion. There have been reports of recovery of proteins after chemical precipitation in potato starch industry waste (21), but the economics of this process often do not justify the recovery.

It will be apparent from the literature discussed above that no coordinated and unified efforts have been made to ascertain the removal characteristics of pure macromolecules of colloidal sizes in activated sludge systems. The works reported by Ruchhoft et al. (22), Placak and Ruchhoft (9) and Gaudy (10) were fragmentary, and they did not study the colloidal substrate removal thoroughly, since this did not constitute the primary purpose of their research.

Further, the mechanism of removal of colloidal components of waste in activated sludge systems has not been clarified. It has been suggested by Eckenfelder and Gloyna (23) that colloidal matter in waste is removed on contact by a physico-chemical process like adsorption on the activated sludge surface. Smallwood (24) experimentally showed that adsorption was the predominant phenomenon in the removal of a colloid, C_{14} -tagged Chlorella algae, in an activated sludge system. However, the substrate used was quite unusual and perhaps not representative of colloidal wastes. Further, the presence of 40 percent of the radioactivity in the supernatant liquid after six hours of aeration does not necessarily mean that the balance of the radioactivity was adsorbed on the cells in the activated sludge system. There is a good possibility that the balance of the radioactivity was incorporated into the cells.

In this context, mention must be made of the modified activated sludge processes like "Biosorption" (25), "Contact Stabilization" (26), and "Bioflocculation" (27), which are largely dependent on the high initial removal characteristics of the particular waste and the sludge. The mechanisms proposed for these processes are essentially the same as those mentioned earlier in the case of the conventional activated sludge process. These processes are applicable only in cases where a very high percentage

(70-80 percent) of the incoming organic matter is sorbed or complexed with the recycled and reactivated sludge in a short contact period. It has been reported by Weston (26) that with filtered and unfiltered sewage, 76 percent and 68 percent respectively of the BOD fed was removed on contact with stabilized activated sludge, thereby indicating a high removal of soluble and colloidal organic matter in sewage. The "Contact Stabilization" process has been successfully applied to textile waste (28) and canning waste (29), both of which contain high proportions of colloidal matter.

The large size of the colloid molecules prohibits their direct entry into the microbial cells where the biochemical oxidation of the organic matter actually occurs. Extracellular enzymes are necessary to degrade these molecules to sizes small enough to cross the cytoplasmic membrane. No reports have been published to indicate the presence or importance of these exoenzymes in degrading the macromolecules in activated sludge systems.

Thus the mechanism of the removal of colloidal substrates in the activated sludge system is still in the theoretical stage without much experimental evidence.

3. SCOPE OF THE INVESTIGATION

From the preceding discussion, it will be evident that there is a great need to obtain precise information about the removal characteristics of colloidal components of a waste in the biological treatment process.

This knowledge would prove invaluable in designing treatment plants for industrial waste with a high colloid content (e.g., textiles, food, paper and pulp). The use of the "Contact Stabilization" process may be appropriate for wastes containing solely colloidal components, if there has been a high initial sorption of colloids on the sludge.

The present investigation was initiated to determine the removal kinetics and mechanisms responsible for removal of a colloidal substrate in an activated sludge system under different operating conditions. The variables which affect the removal kinetics of the substrate in an activated sludge system and which were studied were temperature, food-to-microorganism ratio (F/M), acclimation to various substrates, and anaerobiosis.

The operating variables for the activated sludge system chosen were selected in order to obtain a comprehensive idea about the removal characteristics of the colloidal substrate.

The colloid chosen for study was soluble potato starch, a lyophilic molecular linear colloid. The use of starch in industry is very widespread. Some wastes from the food, paper and pulp, textile and fermentation industries contain either starch or its degradation products. The quantity of starch in domestic sewage is probably negligible, but some other polysaccharides may be present. Starch is an ideal colloid to study in an activated sludge system since it can form a colloidal solution on heating and can be separated easily from the microorganisms by membrane filtration or

centrifugation. Moreover, simple quantitative tests specific for starch are available.

4. THEORETICAL CONCEPTS

4.1 Colloids

Colloids are particles which are so small that they behave in some respects like molecules, and sometimes molecules so large that they behave in some respects like particles. Although colloids generally vary in size from 2 m μ to 100 m μ (0.1 μ), particles larger than 100 m may also behave as colloids (30). (One m μ is equal to 10 angstrom or 10^{-7} centimeters.) Particles having large extensions in one or two dimensions will also exhibit colloidal phenomena. Thus, a particle of 1 m μ thickness and 1 μ length would be considered as a colloid.

Balmat (2) defined sewage colloids as particles between 1 m μ to 1 μ , and used the term supracolloidal for those particles ranging in size from 1 μ to 100 μ . For the purpose of this investigation, the upper size limit of the colloid was defined to include those particles which could be filtered through a 1.20 μ membrane filter. The definition based on size is arbitrary since it entirely neglects the important organization that colloidal matter is known to possess. But from the engineering point of view, it affords a way of distinguishing a colloidal particle from smaller molecules. According to this definition, a bacterium is also a colloidal particle.

Colloids have been classified in various ways. Depending on the stability of their dispersed state in a medium (which is often called sol in colloid chemistry), colloids have been defined as lyophobic or lyophilic (31). Lyophobic sols are those in which no affinity exists between the particle and the solvent. If the solvent is water, they are said to be "hydrophobic." The stability of these sols is mainly dependent upon the charge of the particles. Lyophobic sols are not very stable. In lyophilic

colloids, there is an interaction between the particle and the solvent, one combining with the other and becoming solvated. Most organic colloids are lyophilic. Groups which have a tendency to bind water are called hydrophilic groups. The $-OH$ group in hydroxides and polysaccharides, as well as the $-COOH$ and $-NH_2$ groups in proteins are examples of hydrophilic groups.

What are the properties which make the colloidal particle different from other particles?

Firstly, colloids pass through simple filter paper, but may be retained on ultrafilters (10-200 μ), whereas coarse particulate matter is retained on the former and small molecules pass through the latter. Thus, particle size is a very important difference between colloidal and non-colloidal systems. Viscosity is one of the most important properties of a colloidal system. While the viscosity of lyophobic systems differs slightly, if at all, from that of the dispersing medium, the viscosity of lyophilic systems is much greater than that of the dispersing medium. The reason for this behavior becomes apparent when the nature of the particles composing the internal phase of lyophobic and lyophilic systems is compared. The lyophilic colloids are, without exception, substances which become solvated with the dispersing medium and this property accounts for their viscosity. The viscosity of lyophilic sols depends on their particle shape. Linear colloids have viscosities 10-100 times higher than spherocolloids.

The diffusion coefficient of a colloidal particle is low because of its large size, and only the smallest particles can be dialyzed at a very low rate. Colloidal solutions exert a very low osmotic pressure. This peculiar property of colloids is easy to understand. The colloidal particles cannot penetrate any membrane, so the solvent will diffuse in to dilute the colloidal solution resulting in the osmotic pressure. Moreover,

according to Van't Hoff's Law, the larger a molecule, the lower will be its osmotic pressure for any given weight concentration, as osmotic pressure is a colligative property.

Interfacial reactions and events are of paramount importance in colloidal systems. A colloidal system has two kinds of interfaces -- firstly, the macroscopic interface which the solution shares with air (gas/liquid), and with the walls of the container (liquid/solid), and secondly, the submicroscopic interface in a colloidal solution between the colloidal particles and the solvent. Since organic colloidal solutions are mostly hydrophilic sols, they have a low surface tension. When a protein is dissolved in water, it lowers the surface tension moderately. A much greater effect is achieved by surface active substances such as soaps, saponins and detergent substances; on the other hand, substances like commercial starches have very little influence on the surface tension of the solution.

It is a well known fact that, with an increasing degree of dispersion of a substance, an enormous increase in the extent of the surface area occurs. Adsorption is primarily a colloidal phenomenon because it is only in colloids that we find materials with sufficient area to adsorb appreciable quantities of another substance. Adsorption occurs at surfaces to lower the free energy of the surface. The property of a colloid is often dependent on the nature of the adsorbed layer. The adsorption phenomenon is discussed in detail later in this chapter.

Among the optical properties of colloidal solutions, opalescence is very characteristic. This phenomenon is a result of scattering of light of short wave length (producing the sensation of blue and violet) by the colloid particles, whereas light of long wave lengths (yellow, orange and

red) passes undisturbed through the sol (31). Opalescence depends mainly on particle size. Many colloidal solutions are completely clear, but if a sharp intense beam of light is passed through such clear sols, the light path appears turbid. This is known as the Faraday-Tyndall effect and the reason for its occurrence is similar to that for opalescence. Even small molecules can scatter light, although the amount scattered is quite small. The lyophilic linear colloids (cellulose, starch and gelatin) exhibit a weak Tyndall effect. This indicates that fibrous particles, mostly those of organic molecular colloids, scatter the light comparatively little. The scattering depends on the difference in refractive indices between the solvent and particles, as well as the structure of the particles, their size and their solvation.

From time to time, reference has been made to soluble colloids or colloidal solutions. Any colorless particle dispersed in a liquid smaller than the wave length of ordinary visible light, 400 mμ to 750 mμ, may not be seen by eye or even with the ordinary microscope but for the scattering of light by the particle. Such a solution may be clear to the naked eye, and there may not be any affinity of the particle to the solvent resulting in a nonsolvated particle. However, there are macromolecular solids which form true colloidal solutions when the affinity of the macromolecule for the solvent molecule is greater than the cohesive force and secondary bonds acting between the macromolecules themselves in the solid phase. In the case of potato starch and water, heat is necessary to break the bonds of the solid granule of starch in order to obtain a solution. The affinity of a certain solvent for the macromolecule depends on the chemical composition of both. If the macromolecules are composed of lipophilic groups such as CH_3^- , $-\text{CH}_2^-$, $-\underset{|}{\text{CH}}^-$, and C_6H_5^- , they will dissolve in benzene, heptane,

chloroform and similar solvents. However, they will not dissolve in water. On the other hand, the proteins which contain many hydrophilic groups ($-\text{COOH}$, $-\text{NH}_2$, $-\text{NH}-$, $=\text{CO}$) may be soluble in water, but are insoluble in benzene, toluene or ether. If the macromolecular substance is to dissolve, it must become solvated by the molecules of the solvent. The latter penetrates the bulk of the solid polymer and sticks to the macromolecule. Because of the Brownian movement the solvated macromolecules are then eventually pushed off into the solvent.

To summarize, colloids are large molecules or small particles, ranging in size from a few millimicrons to one micron. In the dispersed state they possess very characteristic properties which may be quite different when compared with those of small molecules or large particulate dispersions.

It must be borne in mind that the distinction of colloids from other states of matter based on particle size is arbitrary, and probably there are many instances where exceptions to the generalization exist.

4.2 Removal of Substrate in Biological Systems

4.2.1 BOD Removal by Biological Sludges (23)

In order to understand the kinetics of removal of organic substrates, it would be pertinent to discuss first the BOD removal by the biological sludges. In biological systems BOD removal may be considered to occur in two stages, an initial high removal of suspended and soluble BOD, followed by a slow removal of the soluble BOD. The initial BOD removal of the various components of the waste in the biological system has been attributed to removal of suspended matter by enmeshment in the

biological floc, removal of colloidal materials by physico-chemical adsorption on the biological flocs, and a biosorption of soluble organic matter by the microorganisms. The removal of suspended matter will be rapid and dependent upon adequate mixing of the waste with sludge. There is some doubt as to whether this initial removal is the result of enzymatic complexing or a surface phenomenon, and whether the organic matter is held to the bacterial surface or within the cell as a storage product, or both. The amount of immediate removal of soluble BOD is directly proportional to the concentration of sludge present, the sludge age and the chemical characteristics of the soluble organic matter.

These three reactions occur immediately on contact of sludge with the waste. The colloidal and suspended material must undergo sequential breakdown to smaller molecules in order that they may be made available to the cell for synthesis. There is some doubt as to the exact mechanism responsible for the high initial removal of soluble organic matter. It is believed that the organic matter in the soluble form is initially removed from solution as a surface complex or by an enzymatic transport system leading to a cellular storage product (23). This material is later used by the cell for synthesis when the external substrate is exhausted. The nature of the storage product varies with the characteristics of the substrate used but lipid and carbohydrate polymers have been shown to be the most predominant storage product in the cells (32). At a high concentration of BOD, the rate of synthesis is less than the rate of surface sorption, resulting in a constant and maximum rate of cellular growth. With the decrease in external BOD concentration, a point is reached when the rate of uptake of substrate will be equal to the rate of synthesis of the cell. With continued aeration, the rate of uptake of substrate will decrease with decreasing concentration of external BOD, which in turn will

limit the rate of sludge synthesis. Although the uptake of substrate by the cell decreases progressively with time due to a decrease in the external substrate concentration, synthesis continues at a maximum rate until the storage carbon concentration is depleted by conversion into cellular protoplasm. The accompanying respiration causes a decrease in cellular mass and cellular carbon, accompanied by an increase in cellular nitrogen (33).

The oxygen utilization per unit of cell mass remains constant at a maximum rate during the log growth phase since substrate is not limiting and the rate of synthesis controls the overall rate of oxygen utilization. The oxygen utilization continues at a maximum rate beyond the log growth phase until the stored carbon is depleted. Then the oxygen uptake rate decreases as the rate of BOD removal decreases (34).

4.2.2 Kinetics of Substrate Removal

There have been several attempts to formulate mathematical relationships to explain the organic substrate removal in a biological system. Among them, the two phase theory (35), the Michaelis-Menten formulation (36), and the linear removal concept (37) have found the widest acceptance.

The two phase theory states that the rate of organic substrate removal per unit cell remains constant to a limiting concentration (log growth phase) below which the rate becomes dependent on concentration (declining growth phase) and then decreases. The rate of cell growth may continue at a maximum rate longer than the rate of organic substrate removal due to the utilization of the stored matter. The rate of organic substrate removal during the declining phase may follow first order kinetics,

or in the case of a complex waste, a retardant reaction as various components are removed at different rates. The log growth phase is defined as:

$$\frac{dS}{dt} = kS \quad 1$$

where, S = biological volatile suspended solids, mg/l

k = sludge growth rate, per hour (natural logarithms)

A fraction of the substrate removed is converted to cells if there is no storage material and no limitations of nutrient and oxygen.

$$\Delta S = a (L_o - L_t), \text{ or } \Delta S = a L_r \quad 2$$

where, ΔS = sludge synthesized from the utilized substrate

L_o = substrate concentration at start, BOD, or COD

L_t = substrate concentration at time t , BOD, or COD

L_r = substrate removed in time t , BOD, or COD

a = fraction of the substrate utilized (BOD or COD) converted to cells. (The factor converting substrate BOD or COD mg/l to cell protoplasm mg/l is included in the factor "a.")

Then Equation 1 resolves to

$$\frac{a}{S} \frac{dL}{dt} = k \quad 3$$

which, on integrating, gives

$$\log (1 + aL_r/S_o) = k_1 t \quad 4$$

where S_0 = initial sludge concentration, mg/l; $k_1 = \frac{k}{2.303}$

The plot of $\log (1 + aL_r/S_0)$ versus t gives a straight line whose slope defines the logarithmic growth constant k_1 (in common logarithms).

In the case when $\Delta S < S_0$, little error is introduced by letting $S = S_a$, the average sludge concentration over the range under consideration. Equation 4 becomes

$$k_1 = aL_r/S_a \quad t \quad 4a$$

Equation 4a indicates that the removal rate, expressed in milligrams substrate per hour per gram sludge will be approximately constant (23).

Below a limiting concentration level, the sludge growth rate k , and hence the substrate removal rate, become concentration dependent.

$$\frac{dL}{dt} = -k_2 L \cdot S_a \quad 5$$

which on integration yields

$$\log \frac{L_0}{L_t} = k_2' S_a \quad t \quad 6$$

where k_2' = logarithmic substrate removal rate when the sludge growth rate becomes concentration dependent (common logarithms).

It has been reported (34) that even after long periods of aeration, a small residual BOD or COD remains, since autooxidation of the sludge results in the utilization of cellular materials and release of end products of metabolism and respiration which are resistant to biooxidation.

The two phase theory discussed in the preceding paragraph results in a discontinuous function (36). The Michaelis-Menten relationship overcomes this shortcoming and yields a continuous curve. This equation can be written as (38):

$$\frac{dS}{dt} = k S \quad 7$$

$$\text{where } k = K_e \frac{L_t}{L_k + L_t}$$

L_k = Michaelis-Menten constant

K_e = maximum removal rate when concentration is not limiting.

In terms of concentration units

$$\frac{a}{S} \frac{dL}{dt} = K_e \frac{L_t}{L_k + L_t} \quad 8$$

At high substrate concentration, $L \gg L_k$, Equation 8 approaches Equation 3, while at low concentration of BOD, $L \ll L_k$, Equation 8 approaches Equation 5.

For simplicity, Equation 8 can be restated as follows: $\frac{dL}{dt}$ can be written as v and, if S is kept constant, then

$$v = \frac{V L_t}{L_k + L_t} \quad 9$$

where V = the maximum reaction velocity attained when L_t is large.

This equation can be rearranged in several ways to give straight line plots from which V and L_k can be determined.

Wuhrmann (37) showed that the rate of removal of pure compounds in the activated sludge system was linear with time to a very low concentration. The kinetics of these reactions were explained by the laws

governing enzymatic reaction. The use of nonspecific measures of the substrate concentration, such as BOD, organic nitrogen, etc., and the complex mixture of wastes with widely varying reaction rates usually resulted in a first order or retardant reaction forms.

The application and extension of the above-mentioned principles (which have been derived usually for simple organic substrates) to the colloidal substrates could yield an understanding of the kinetics of the process. As mentioned earlier, a colloid has to be degraded into smaller components by exoenzymatic action before it can be utilized by the cells. The removal rate of these breakdown products of the colloid may differ, depending on the nature of the individual product. The removal of a mixture of these smaller components from the colloid, which are removed at different rates, as measured by a nonspecific test, BOD, COD, etc., may be linear until the most readily removable substance is completely removed, followed by a decreasing rate as the other substances are progressively removed. This decreasing rate may be approximated as a first order or a retardant reaction.

4.2.3 Temperature Effects on Biological Treatment

Temperature is an important variable which affects the performance of biological treatment. Various investigators (39)(40)(41) have studied the effect of temperature on aerobic biological treatment processes. Schroepfer et al. (39) observed in a trickling filter that temperature effect was of decreasing importance as the organic load to the filter was increased. Intermittent dosing had temperature effects on the BOD removals as it changed the temperature of slime more than continuous operation of a filter. It was

proposed that, based on the temperature of slime rather than that of the waste, the effect of temperature on the BOD removal efficiency of a filter was constant, regardless of the organic load.

Ludzack et al. (40) using a continuous activated sludge unit found that temperature had a significant effect on the variety and motility of the predator population. The waste removal at 30°C was 10 percent higher than at 5°C, although the solids accumulation per unit weight of BOD input was substantially greater at 5°C than at 30°C. Keefer (41) reported operating results over a 20-year period of a municipal activated sludge plant. The variation of average percent BOD removal with temperature was dependent on the flow through the plant and increased as the organic load was increased. On the other hand, Okun (42) reported little effect of temperature on BOD removal in activated sludge systems between 8-35°C. Sawyer, Frame and Wold (43) studied the temperature fluctuations in an activated sludge process and concluded that the adaptation of the activated sludge process to temperature change was immediate.

Although the bacterial cell is a very complex entity, it is logical to assume that reactions of the cell follow the laws of thermodynamics. The variation of the biological reaction rate constant K can be expressed, therefore, by the Van't Hoff-Arrhenius relationship (34):

$$1/K \frac{dK}{dT} = \frac{\Delta E}{RT^2} \quad 10$$

where R = universal gas constant

T = absolute temperature

K = reaction rate constant

ΔE = energy of activation, a constant

Integrating

$$\log K = - \frac{\Delta E}{2.3 RT} + C \quad 11$$

or

$$\log K^1/K^2 = \frac{\Delta E (T_2 - T_1)}{2.3 R T_2 T_1} \quad 11a$$

A plot of $\log K$ versus $1/T$ produces a straight line with a slope $-- \Delta E/2.3 R$ and ΔE for activated sludge has been reported to be 14400 cal per mole (34).

Another common way to express the effect of temperature on a biological system is by the use of the term Q_{10} or "temperature coefficient." The temperature coefficient is simply an expression of the number of folds increase in the rate of a process for each 10-degree rise in temperature.

The temperature influence on biooxidative processes has been observed to be less than estimated by the foregoing equation. Since all processes represent a sequence of related reactions (oxygen transfer, absorption, oxidation, etc.), it is probable that temperature effects on oxidation are not the controlling reactions under all temperature conditions. Oxygen transfer or respiration may be the critical factor at other temperatures.

4.2.4 Acclimation

Artificial and natural purification processes are accomplished by the combined activities of a number of microbial species and variants. The active members in a particular system are dependent on the composition of the substrate, i.e., both organic and inorganic components, pH, and temperature, and have been selected and adapted from the infinite number of microorganisms and microenvironments existing in nature. Selection is

accomplished by the fortuitous matching of potentially competent microorganisms of the habitat and the available waste materials (44).

Further adaptations are accomplished by producing the necessary new enzymes, either through activation of latent characteristics, or through genetic changes that arise spontaneously or by stimulation from environmental factors.

Mutations may produce variants, either more or less suited to the environment, and only the more suited progeny are likely to survive. The parts played by recombination, transformation and transduction in the selection of the microbial biota in a waste treatment process may be important but have not yet been fully evaluated (44).

Enzyme induction is a physiological change in all of the cells rather than a genetic change in part of the bacterial population. Enzyme induction and repression have been shown to be related through the regulator genes of the microorganisms (45). The regulator genes are claimed to be responsible for producing a regulator molecule termed the cytoplasmic repressor. In a typical repressible system, the cytoplasmic repressor is assumed to be incomplete and active in repressing enzyme synthesis only when combined with low molecular weight corepressor molecules. In a typical inducible system, the cytoplasmic repressor is considered to be fully effective and able to prevent enzyme synthesis until it is rendered inactive by combination with inducer molecules.

It has been observed by many investigators (46)(47) that acclimation of activated sludge to the substrate increases the rate of biooxidation. In fact, many toxic compounds like phenols and cyanides have been successfully treated biologically when a proper acclimation of the microorganisms has been achieved.

4.2.5 Mechanism of Removal of Substrate in Biological Systems

Earlier works with the conventional activated sludge process (48) and later works on a modified activated sludge process (25) seemed to indicate that on contact with some waste, there is an appreciable waste removal in a relatively short time followed by a progressively slow removal. The initial high removal of soluble simple organic compounds has been attributed by some investigators (9)(49) to adsorption of the waste by the sludge. Katz and Rohlich (50) studied the adsorption characteristics of activated sludge using settled sewage and glucose as substrates and obtained a relationship following a modified Freundlich adsorption relationship.

After extensive experimentation with soluble simple organic compounds, Wuhrmann (37)(46) concluded that the removal of these substances from an activated sludge system was due to the physiological reaction of the sludge organisms and that physico-chemical processes, like adsorption, played no part.

Smallwood (24), using glucose and nutrient broth as substrates, found that enzymatic assimilation was the predominant factor, and adsorption was not perceptible. He used sodium azide as an inhibitor of assimilation processes and obtained no buildup of sludge solids with the substrates fed. If adsorption was the significant mechanism in the purification process, the azide should have made no difference in the buildup of the sludge solids.

At the present time, many investigators believed that the removal of soluble simple organic matter occurs by enzymatic assimilation. The simple organic molecule is usually transported by a specific enzymatic permeation process across the permeability barrier, the cell membrane, into the cell. The utilization of the substrate occurs inside the cell which involves synthesis of new protoplasm and production of energy (respiration)

for cell maintenance and synthesis. The end product of the reactions may be excreted back into the medium.

The specific permeation process of simple organic and inorganic molecules into the cell has recently been elucidated for specific compounds in certain cells (51). It is believed that these enzymes, permeases, are specific for a particular metabolite and are distinct from the metabolic enzymes. Although the permease system for many substrates has been defined down to the genetic level, none of these enzymes has been isolated or analyzed in a cell-free state. In some cases it has been found that the rate-limiting step of the utilization of a substrate was involved with the permease system. It has been proposed that permease systems provide a means of regulatory mechanism for growth, substrate utilization, enzyme induction and repression, and feedback inhibition of biosynthetic enzymes.

In the case of colloidal macromolecular substrates, the mechanism of their utilization by microorganisms would be different. It has been shown with pure culture studies, that enzymes are secreted by the microorganisms outside the cytoplasmic membrane; here, the macromolecules are degraded into smaller sizes, which are capable of being transported across the permeability barrier. Most exoenzymes liberated outside the cell are concerned with the metabolism of very large molecules, with some exceptions like penicillinase. The breakdown of these large molecules by the exoenzyme may be the rate-determining step and then the kinetics of uptake of the large molecules will depend on this first reaction. Then again, the products of the enzyme action may also be macromolecular, and may be attacked further by the same enzyme. The smaller molecules formed after exoenzymatic action on the colloid may have a different rate of permeation into the cell. Thus, the overall process of macromolecular degradation by

microorganisms is quite complex.

In some cases, the exoenzymes may be located on the cell surface instead of in the medium containing the cells. The colloidal substrate would then be physically adsorbed on the cell and broken down to smaller sizes by the surface-located enzyme. Of course, the adsorption of the colloidal substrate need not occur exclusively on cells possessing surface-located exoenzymes. The process of adsorption of the substrate on the cell depends on the physico-chemical properties of the system and perhaps is a physical process. The degradation of macromolecules in the medium by intracellular enzymes liberated due to cell lysis has also been reported (52). The above is a possibility in any microbial system, and it is quite difficult to define an enzyme as endocellular or exocellular without measuring the extent of lysis in the medium.

The breakdown of large molecular weight substrates in biological systems could be attributed to the following mechanisms:

a. An exoenzyme in the medium influences the degradation of the substrate into molecules of smaller size; no adsorption of the substrate occurs.

b. An exoenzyme exists in the medium, and a portion of the substrate is adsorbed on the cell; a breakdown reaction of the large molecules into smaller fragments occurs in the medium, and the amount of substrate adsorbed on the cell decreases as the equilibrium shifts towards desorption.

c. There exist both cell-surface-bound enzyme, and an exoenzyme in the medium, but none of the substrate is adsorbed on the cell. When the substrate molecules collide with the cell-surface-located enzyme, they undergo degradation. Apart from this, the surface-located enzyme does not

appear to be of much use.

d. In this case also, cell-surface-bound enzyme and exoenzyme exist. A part of the substrate is adsorbed on the cells, and the remainder exists free in the medium. The surface-bound enzyme is responsible for the degradation of the cell-adsorbed portion of the substrate, while the exoenzyme attacks the rest of the substrate in the medium.

e. The exoenzyme is completely bound to the cell surface, whereas the substrate is only partly adsorbed on the cell, the rest being present in the medium. The surface-located enzyme acts upon the adsorbed substrate, and gradually, the substrate molecules from the medium begin to replace those already degraded from the adsorption sites.

f. Again, the exoenzyme is wholly bound to the cell surface; there is no adsorption of the substrate on the cell at all. In such a case, the breakdown of the substrate is dependent upon chance collision between the molecules and the enzyme at the sites of the cell at which the enzyme is located.

4.3 The Structure, Properties and Biodegradation of Starch (53)

4.3.1 Structure and Properties

The reasons starch was chosen as the substrate for this study have already been mentioned. A discussion of some of the chemical and biochemical properties of starch would be appropriate at this stage.

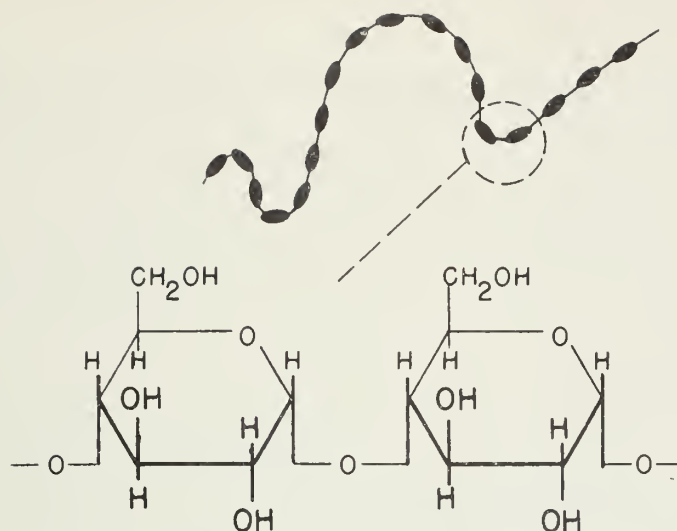
Starch is a glucan stored as a food reserve in the form of polysaccharides in plants. The insoluble particles of the polysaccharide are laid down as starch granules. The size and shape of the granules depend on the botanical source from which they are isolated. Relatively little is

known about the detailed structure of the starch granule. Microscopically, the granule appears to be made up of a series of concentric lamellations. The molecules in each granule are arranged in an ordered radial manner for the granule exhibits birefringent properties, and the nature of this phenomenon is not quite clear. The starch granule is insoluble in cold water; on heating, it swells in size, reversibly at first, until the birefringent properties disappear at the "gelatinization temperature." After this stage has been reached, further heating causes the granules to disrupt and form a "dispersion." A starch dispersion contains at least two chemically distinct species, i.e., amylose and amylopectin.

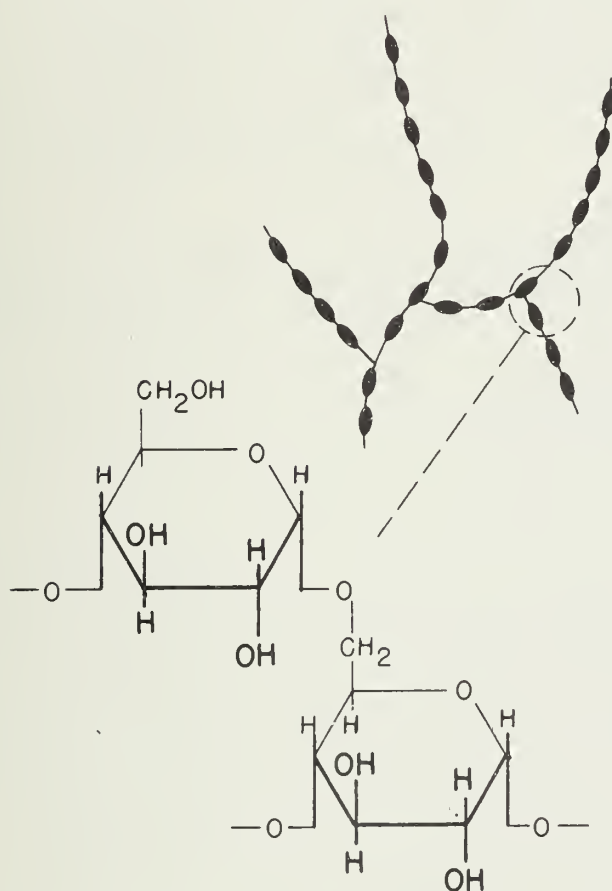
Amylose is essentially a linear structure of several thousand α -D-glucopyranose units linked by α -1,4-bonds; amylopectin is a much larger molecule with a highly branched structure in which chains of α -1,4-linked glucose units are joined through α -1,6-bonds (Figure 1) (53). In general, starch granules contain more amylopectin than amylose. Evidence indicates that approximately 5-10 percent of the granule consists of a substance which has properties intermediate of the two components (54).

The flexibility of the 1,4-bonds of amylose enables the molecules to attain various conformities. The helical form is one of these and is considered to be responsible for some chemical reactions, e.g., the staining reaction with iodine, and complex formation with polar organic molecules, the complex held within the helix. As a matter of fact, the helical form occurs only in solution in the presence of a complexing agent. Without the latter, the molecule attains a random coil in solution.

The average length of branches in the amylopectin molecule varies from 19 to 26 glucose units. Amylopectins contain bound phosphorus, which is believed to exist as ester phosphate on the primary alcoholic group. It



A. LINEAR AMYLOSE MOLECULE



B. BRANCHED AMYLOPECTIN MOLECULE

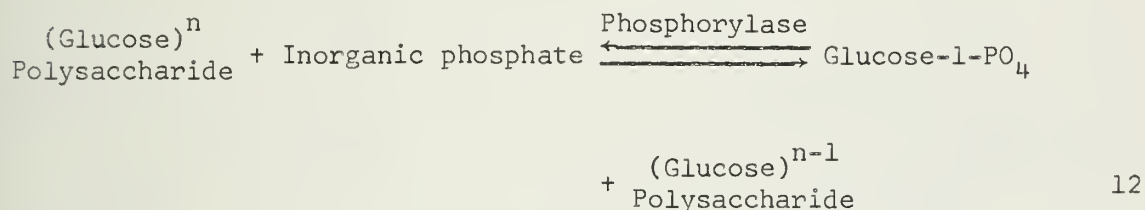
FIGURE I. STRUCTURE OF AMYLOSE AND AMYLOPECTIN MOLECULES
(53)

is of interest that, although the phosphate group is present in a very small ratio to the glucose molecules (1 per 100-300 glucose residues), it makes a profound difference in the physical behavior of the polysaccharide in aqueous solution (55).

4.3.2 Enzymatic Decomposition

There are 2 classes of enzymes which can act upon starch components. The more important of these are termed amylolytic, and consist of hydrolytic enzymes, like α -amylases, β -amylases, amyloglucosidases.

The second group includes the phosphorylases, and their action can be represented by the general equation,



Amylolytic Enzyme Action

The nomenclature of α - and β -amylase arises from the mutarotation of the products. α -amylases can be detected in animals, plants, fungi and bacteria. The action pattern of these enzymes has been thought to be involved with the random degradation of α -1,4-linkages in high molecular weight starch materials, though evidence for nonrandom degradation has also been presented (56). The action of α -amylase on amylose is characterized by a rapid loss in viscosity and iodine stainability, accompanied by an increase in reducing power, all of which could indicate random scission of 1,4-bonds. This is shown in Figure 2 (53).



A. RANDOM DEGRADATION OF
AMYLOSE BY α AMYLASE



C. STEPWISE DEGRADATION OF
AMYLOSE BY β AMYLASE

● GLUCOSE UNITS IN MAIN CHAIN

○ NONREDUCING GLUCOSE AT
ENDS OF CHAINS

+ POINT OF AMYLASE ATTACK



B. RANDOM DEGRADATION OF
AMYOPECTIN BY α AMYLASE



D. STEPWISE DEGRADATION OF
AMYOPECTIN BY β AMYLASE

FIGURE 2. SCHEMATIC DIAGRAM SHOWING AMYLASE ACTION (53)

The action of α -amylase on amylopectin is comparable, a random scission of α -1,4-bonds occurs as indicated in Figure 2, while the α -1,6-branching points are unaffected. The viscosity and iodine stainability undergo a decrease in activity, and again, as in amylose, there is also an increase in reducing power.

β -amylase can be found in plant materials like cereals, soyabeans and sweet potatoes. The enzyme degrades any linear chain of α -1,4-linked glucose residues from the nonreducing end with the stepwise formation of maltose as shown schematically in Figure 2.

The action of β -amylase on amylose is complicated. If amylose was a simple 1,4-linked glucan, then on hydrolysis, a complete conversion to maltose would be expected. However, with amylose obtained from a starch dispersion, this is not to be found. The conversion to maltose is to the extent of 64-84 percent of the amylose. It is now generally accepted that this incomplete conversion is due to some modification in the structure of amylose. When β -amylase acts on amylopectin, it degrades the molecule from the nonreducing ends of all the outer chains until the action ceases at a point which is located about two or three glucose units from the branching point. 56-58 percent of the structure can be digested in this manner, leaving behind a high molecular weight dextran.

Two other starch degrading enzymes are of interest. Amyloglucosidase from Aspergillus niger breaks down starch with the liberation of glucose in a stepwise manner; in some way not quite understood, the enzyme appears to bypass the 1,6-branching point (57). The commercial production of high quality crystalline dextrose is currently based on this reaction. Bacillus macerans produces an enzyme which degrades starch to oligosaccharides called the Schardinger dextrans.

Phosphorylase

Phosphorylase acts reversibly on α -1,4-linkages of sugars.

Substrates for this enzyme include starch and glycogen. The degradation of amylose by phosphorylase is an equilibrium reverse reaction. If the removal of glucose-1-phosphate formed is coupled to the degradation of amylose, the reaction proceeds to completion.

The breakdown of amylopectin or glycogen stops far short of completion, with the accumulation of a polysaccharide resistant to further attack by phosphorylase. This polysaccharide is a limit dextrin. Phosphorylase activity can proceed only from nonreducing ends to branch points.

A specific 1,6-amyloglucosidase has been found in some systems, which can hydrolytically degrade the branch points. This enzyme, together with phosphorylase, can degrade amylopectin and glycogen completely to glucose-1-phosphate.

Among the enzymes mentioned above, only α -amylase has been found to be extracellular in microbial systems (58). In an activated sludge system utilizing starch, α -amylase is perhaps the most important enzyme to be considered.

4.3.3 Biological Degradation

The amylolytic and phosphorolytic action on starch yields glucose, oligosaccharides and glucose-1-phosphate. When these products are transported inside the bacterial cell, they can be utilized in a number of ways. They can be metabolized by various routes, like the Embden Meyerhof, Entner Doudoroff, and the Hexosemonophosphate pathways to form pyruvic acid. Pyruvate may enter the citric acid cycle, and result in the formation of

energy and intermediates necessary for the synthesis of new protoplasm. There is also evidence that, initially, part of the carbohydrate may be stored up inside the cell as storage products (lipid, polysaccharide), especially in cases where the initial uptake of the substrate is much faster than the rate of synthesis within the cell. The stored product may be utilized when the exogenous substrate has been depleted (33).

4.4 Adsorption

The adsorption of substrates on cells has been dealt with briefly in the preceding paragraphs. The subject of adsorption is complicated and interesting enough to warrant its discussion in some detail.

Nearly all surfaces have attractive forces for molecules of gases and solutes, and will concentrate the substance on their surfaces. This process is called "adsorption." Adsorption on solid surfaces plays a very important role in colloid chemistry. The surface of most colloids are covered with an adsorbed layer which determines their properties.

The phenomena of adsorption are so diverse that it is difficult to find simple relationships in spite of the voluminous literature on the subject. Consequently, a quantitative treatment of the phenomena is difficult and sometimes impossible. For these reasons, one is frequently restricted to a qualitative treatment of adsorption.

The poor reproducibility of this process is explained by the fact that adsorption is influenced by many different factors. The pressure, temperature, and concentration of the material to be adsorbed are only a few of the important criteria that must be considered. Variables such as surface area and the chemical and physical properties of the surface, which

are difficult to measure, must also be attended to. The physical properties of the colloid depend on the previous treatment and history of the specimen. The surface may be highly polished, smooth, rough, fragmented, porous, crystalline, amorphous or stressed. The degree of dispersion of the adsorbate and the type of dispersion medium are other problems of significance. Therefore, it is not very surprising that a number of adsorption equations have been developed.

For adsorption in solid-liquid interphases, the Freundlich isotherm is applicable. The Freundlich isotherm is an empirical relationship between the amount of substance adsorbed at different concentrations.

$$x/m = \alpha C^{1/n} \quad 13$$

where x/m = substance adsorbed per gram of adsorbent

α = constant, which is a measure of surface area of the solid phase

C = concentration of the adsorbent in equilibrium with the adsorbate

$1/n$ = adsorption exponent, which measures the intensity of adsorption.

The equation, in linear form, reads

$$\log x/m = \log \alpha + 1/n \cdot \log C \quad 13a$$

Adsorption of colloidal particles on solid surfaces occurs in the same manner as the adsorption of smaller molecules and ions. Both lyophobic and lyophilic colloids may be adsorbed. Generally, the adsorption of lyophilic colloids is irreversible, as the adsorbate sticks rather firmly to the surface of the solids. The behavior of protective colloids (e.g., gelatin) is a good example. This is important industrially, when adsorption

inhibitors are used in order to minimize the rate of corrosion of metals.

It has been mentioned earlier that, in activated sludge systems, adsorption, or some other physico-chemical process, is probably responsible for initial removal of the substrate. Bacteria have been known to adsorb bacteriophages by random collision. Therefore, in a bacterial system, adsorption can play an important part in removing the substrate from the medium since the bacteria possess significant surface area.

5. MATERIALS AND METHODS

5.1 Experimental Systems

The activated sludge cultures for all studies were obtained in two systems. The original source of the sludge will be termed the basic seed unit. Sludge from this chamber was transferred to a batch acclimation unit, where it became acclimated to a particular substrate. The sludge withdrawn from the batch unit was used for final experimentation.

5.1.1 Basic Seed Unit

Metrecal was used as the substrate for this continuously-fed activated sludge unit. The unit has been used earlier by Gaudy (10)(59), and its details are shown in Figure 3. The small continuous flow of Metrecal substrate was controlled by an electrolytic pump. The solution being "pumped" was displaced by the small flow of gases generated electrolytically from acid by a variable D.C. current. The Metrecal fed daily was equivalent to 1000 mg/l of Chemical Oxygen Demand (COD) at a flow rate of two liters a day. The average retention time in the aeration compartment was about 24 hours. From the chemical characteristics of the Metrecal substrate and using the BOD values determined it will be evident that the nutritional requirements for normal conditions as measured by BOD/N and BOD/P were satisfied. But, in order to maintain the pH of the system at pH 7.2, 20 ml of 1 M phosphate buffer per liter of mixed liquor was used in conjunction with the substrate. On analysis of Metrecal, it was found that the five-day BOD value was lower than expected on the basis of the COD value (assuming a reaction coefficient of 0.1, the BOD would be 18400 mg/l). Therefore, ammonium chloride at a concentration of 100 mg/l

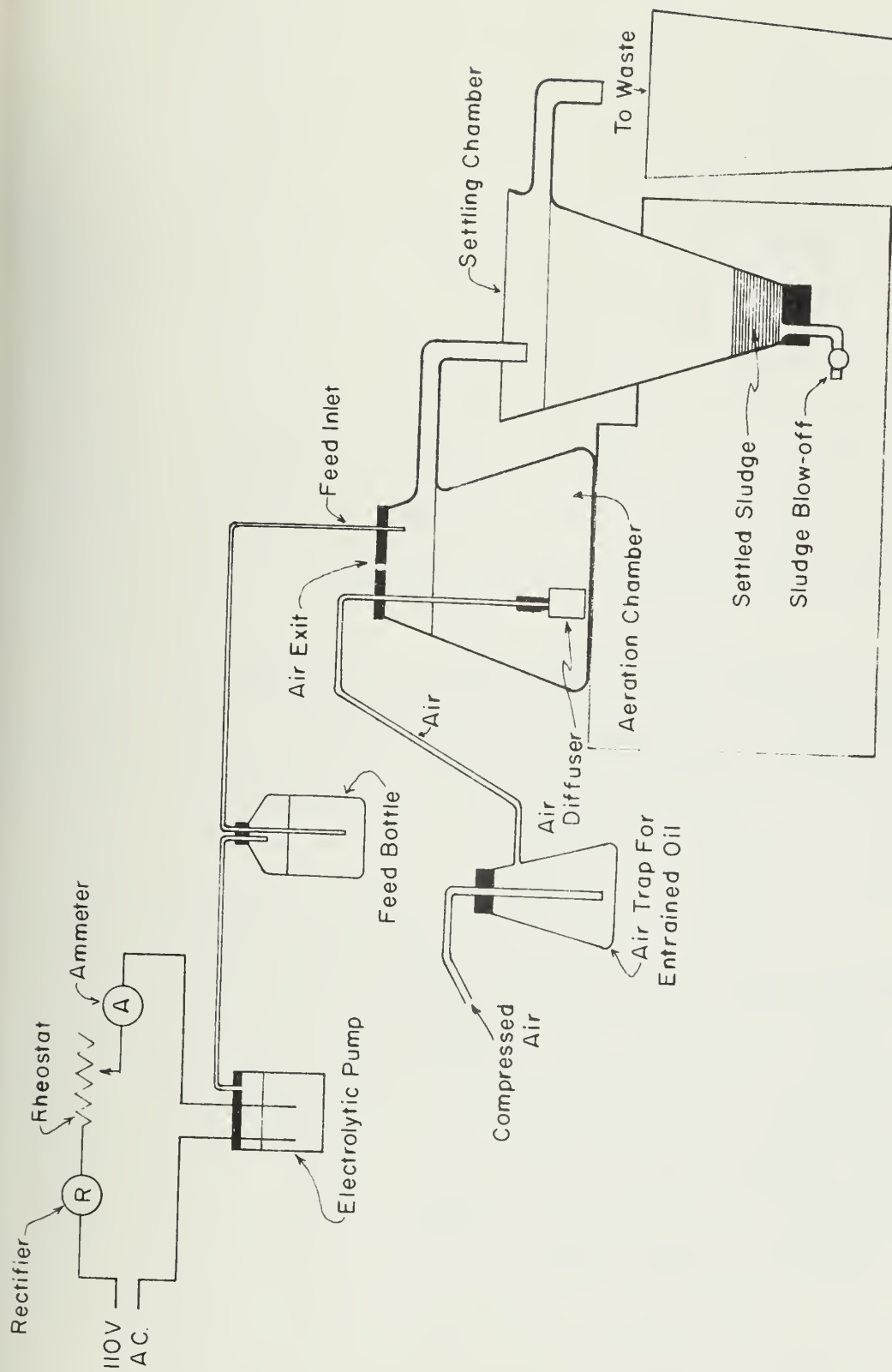


FIGURE 3. BASIC SEED CONTINUOUS ACTIVATED SLUDGE UNIT

was added to the unit to take into account any possible nitrogen deficiency owing to discrepancy in the BOD determination and also the uncertainty of the amount of total nitrogen actually available for cell synthesis. The feed bottle was changed every two days in order to exclude the possibility of microbial growth in it. The air-lift sludge return from the settling chamber, which was planned originally, was discontinued later as it promoted the growth of undesirable filamentous microbial growth in the aeration chamber. When microbial contamination occurred in this manner, the seed sludge from the basic seed unit was not used, and in this case, the seed sludge was taken from a batch-Metrecal-fed unit. The daily COD loadings, the addition of phosphate buffer and ammonium chloride to the batch Metrecal unit were carried out in the same manner as reported for the continuously-fed unit.

The sludge collected at the bottom of the settling tank in the continuous activated sludge unit was wasted every day, or was used as a seed to start the other batch activated sludge units.

5.1.2 Acclimation Units

The seed sludge (500 milligrams) from the basic seed activated sludge unit was used to start every batch acclimation unit of this study. These units were made of glass jars or plexiglass compartments, and had a volume capacity of 1.5 or 2.0 liters. The particular substrate to which the sludge was to be acclimated was used at about 1500 mg/l COD per day (1000 mg/l in the case of bovine serum albumin). These batch units were operated every day in the following manner:

- i. The sides of the units were scraped with a rubber scraper to resuspend the adhering microorganisms after 23 hours of aeration.

- ii. The liquid level lowered by the evaporation of the water was readjusted by adding distilled water.
- iii. A third of the mixed liquor of the unit was wasted prior to the discontinuation of the air supply to the unit.
- iv. The remaining volume of the mixed liquor was allowed to settle for half an hour.
- v. The supernatant was decanted and wasted.
- vi. The unit was replenished with the requisite amount of substrate, ammonium chloride and phosphate buffer.
- vii. The liquid level in the unit was brought up to the original volume with tap water, and the air supply was turned on.

Finally, the unit was aerated for 23 hours before the entire operation was repeated.

Because the source of the air supply to these units was the laboratory air compressor, the air contained some entrained oil; an oil trap was used to facilitate clean air diffusion into the activated sludge units. It was found that no inorganic nutrients, other than nitrogen and phosphorus salts, were necessary for proper operation of these batch activated sludge units, although the use of tap water did satisfy the need of certain trace element requirements.

In the case of the bovine serum albumin fraction V acclimation unit, Dowex Silicone Antifoam had to be used in order to control excessive foaming.

The acclimation of each activated sludge unit was determined by the mixed liquor suspended solids balance of the system under the operating conditions. This balanced state was attained when the unit acquired a "steady state" condition.

5.2 Substrates

5.2.1 Metrecal

In this study, it was deemed necessary to have a constant source of seed organisms for all the batch activated sludge units. The local sewage treatment plant provided a source of activated sludge seed, but it was not used because the period of this study was to be extended over more than a year and it was likely that the type of seed organisms from the activated sludge might change over the entire period, especially in different seasons. Therefore, a continuously fed activated sludge unit was started in the laboratory and maintained throughout the period of the investigation for providing seed sludge. Metrecal was chosen as the substrate for feeding the continuous unit because this nutritionally balanced substrate would be able to support a heterogeneous microbial population in the system. The Metrecal was vanilla flavoured in liquid form. The chemical and biochemical properties of Metrecal are reported in Table 1.

5.2.2 Potato Starch

The properties, structure and biodegradation of starch have been mentioned earlier. Additional properties of potato starch are presented in Table 2. The particular starch used in this study was defined by the manufacturer (J. T. Baker and Co.) as "soluble potato starch for Iodometry." One percent solutions were made by adding a weighed amount of starch in paste form to boiling water and making up the volume in volumetric flasks after cooling. The solution was viscous and had a bluish opalescence. Prior to experimentation, the starch was filtered through a

TABLE 1

PROPERTIES OF LIQUID METRECAL

COD	275,000 mg/l
BOD	92,000 mg/l**
Total Nitrogen	11,610 mg/l*
Total Phosphorus	1,900 mg/l*
Total Protein	7.4%
Total Carbohydrate	11.6%
Total Fat	2.1%
BOD/N	7.92
BOD/P	48.5
BOD/COD	0.334

* Information supplied by Mead Johnson Research Center, Evansville 21, Indiana.

** Settled sewage seed used.

TABLE 2

PROPERTIES OF POTATO STARCH

Granule size range (59)	15-100 microns
Average granule size (59)	33 microns
Amylose, percent (59)	23
Molecular weight (59)	
(a) Amylose fraction	82,000-130,000
(b) Amylopectin fraction	207,000
Chemical Oxygen Demand (COD)(1.00 g/l filtered starch) Average value	1.040 g/l
5-day Biochemical Oxygen Demand (BOD) (1.00 g/l filtered starch) Average value	0.65 g/l

1.2 μ membrane filter so that the size of the molecules present in the solution was less than 1.2 μ . This also allowed separation of the micro-organisms from the substrate at any time during sampling from the activated sludge units.

For non-acclimated sludge studies, glucose and maltose, monomers of the starch polymer were also used as substrates. For the same study, Metrecal and bovine serum albumin, fraction V, were also used.

5.2.3 Inorganic Nutrients

During the entire course of the investigation, the experimental activated sludge units were maintained under conditions whereby there was no deficiency of inorganic nutrients. Ammonium chloride served as the only nitrogen source; Table 3 indicates the COD/N ratios in the acclimation activated and the experimental activated sludge units. The phosphorus source was monobasic sodium phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) and dibasic potassium phosphate (K_2HPO_4), which made up the buffering system (pH 7.2) used in the units. Twenty ml of 1 M phosphate buffer were added per liter of mixed liquor under aeration. The COD/P ratio was quite low because of the large amount of phosphates needed to buffer the activated sludge systems.

Tap water was used to provide the trace metal requirements of the microbial population.

5.3 Analytical Techniques

5.3.1 Chemical Oxygen Demand Test

The Chemical Oxygen Demand (COD) test was one of the key tests employed for the determination of substrate utilization in the activated

TABLE 3

AMOUNT OF NITROGEN FED TO THE ACTIVATED SLUDGE UNITS PER DAY

	mg/l NH_4Cl	COD/N
Metrecal Unit	100	21*
Potato starch, glucose, and maltose units	400	14
Bovine Serum Albumin Unit	-	20**

* Calculated after taking into account organic nitrogen present in Metrecal, of which 50 percent was assumed available.

** Calculated on the basis of 15 percent total nitrogen in protein, of which 50 percent was assumed available.

sludge systems. The procedure as reported in the Standard Methods for the Examination of Water and Waste Water, eleventh edition (61), was used in the modified form (62). The modification eliminated the chloride ion interference by the use of mercuric sulfate in addition to the silver sulfate catalyst.

5.3.2 Membrane Solids

The mixed liquor suspended solids (MLSS) of the activated sludge units representing the microbial cell mass in the system was determined by the membrane filtration method as described by Winneberger, Austin and Klett (63), this being a modification of the original method devised by Engelbrecht and McKinney (64). They reported that the use of individual desiccators for drying the membrane filters proved more advantageous than using a large desiccator. This was also found to be the case, during this study. In order to allow the separation of the colloidal substrate and the cells, membrane filters, 1.2 μ in size, were used. In activated sludge systems, most of the microorganisms were in the form of aggregate flocs and could therefore be retained on filters having a pore size of 1.2 μ . The stainless steel membrane filtration apparatus (47 mm in diameter) and the membrane filters were supplied by the Millipore Filter Corporation, Bedford, Massachusetts.

5.3.3 Starch-Iodine Test

Iodine reacts with starch to give a deep blue color. It has been proposed that the iodine molecules are held strongly in the center of the helical amylose fraction of the starch molecules giving the typical

blue color, which has an absorption peak near 625 m μ . The blue color measures amylose molecules having a degree of polymerization greater than 40 (65). The amylopectin fraction of the starch does not have a high affinity for iodine and complexes with it to form a red or brown color. Dextrins having less than 6 glucose residues per molecule are uncolored with the iodine solution. Short chain amyloses produce purple or reddish shades with iodine.

The quantitative test for analyzing starch in the membrane filtrate of the activated sludge units was performed in a manner similar to that described by McCready et al. (66), as described in the Appendix A.

5.3.4 Total Carbohydrates-Anthrone Test

It was necessary in some cases to determine the total amount of carbohydrates present in activated sludge units which had been fed starch. This information, together with the amount of starch present, would indicate the amount of starch breakdown products in carbohydrate form. Total carbohydrates were determined by the anthrone test. This involves the acid dehydration of the carbohydrate to form the furfural derivative. The latter is condensed with the anthrone reagent to form a green colored compound, which can be assayed spectrophotometrically. Polysaccharides, like starch, are hydrolyzed before the furfural derivative formation occurs in the acid medium of the test. The procedure followed was similar to that reported by Colowick and Kaplan (67) and has been discussed thoroughly by Scott and Melvin (68). Details of the anthrone test are described in the Appendix.

It was found that there was a close correlation between the

anthrone and COD tests, as seen in Figure 4. It will be noticed that the straight line in this figure cuts the COD axis not at the origin but at a positive value. This can be explained by the fact that when the carbohydrate substrate has been completely removed, in an activated sludge system, there still exists a residual COD, which is attributed to stable metabolic products.

5.3.5 Folin-Ciocalteu Test

The determination of the total protein content of the membrane filtrate was made by the Folin-Ciocalteu method. There are several advantages in favor of this method when compared to the Biuret test for protein. Firstly, this procedure is very sensitive, which permits use of small samples (as little as 5 micrograms of protein can be analyzed). Moreover, there are very few compounds which interfere with this test as opposed to the Biuret test (67).

The principle of the test involves the Biuret reaction of the protein with the copper ions under alkaline conditions and subsequent reduction of the phosphomolybdic-phosphotungstic reagent by the tyrosine and tryptophane present in the treated protein. The procedure used was that of Orme-Johnson and Woods (69), which is similar to that reported by Colowick and Kaplan (67), except in the volume of reagents used.

5.3.6 Oxygen Uptake

The oxygen uptake measurement of the activated sludge-substrate systems was made with the Warburg constant volume respirometer according to the methods described by Umbreit et al. (70), only Warburg flasks of

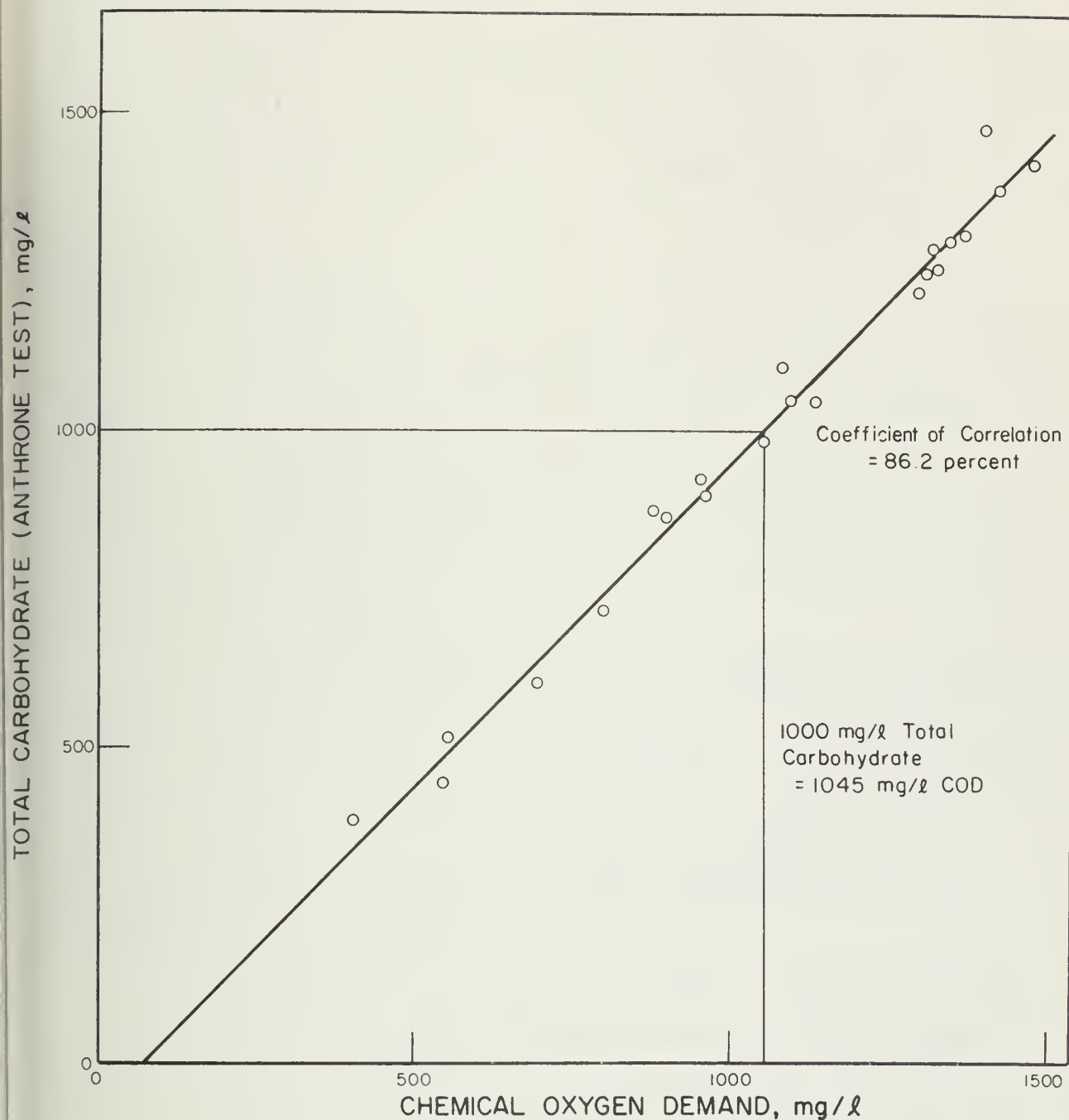


FIGURE 4: CORRELATION BETWEEN TOTAL CARBOHYDRATE AS DETERMINED BY THE ANTHRONE TEST AND COD VALUES FOR POTATO STARCH

125 ml capacity were used. The flask constants were determined by the nitrogen evolution method using potassium ferricyanide, sodium azide, and sodium hydroxide (70). The total volume of the liquid in the flasks varied from 20.5 to 40.5 ml. The flasks were shaken approximately 100 times per minute in a water bath maintained at 20°C throughout the test period.

5.4 Experimental Techniques

The primary purpose of this research was to determine the mechanism and removal characteristics of the chosen colloidal substrate in the activated sludge systems under a variety of conditions. These conditions have been mentioned earlier: namely, acclimation or nonacclimation, different food-to-microorganisms ratios (F/M), anaerobic state under acclimated conditions, and different temperatures.

For studying the removal characteristics of starch under acclimated conditions, seed sludge from the basic Metrecal seed unit was acclimated over a period of seven to ten days to the starch substrate. Acclimation was indicated by the attainment of steady state conditions. Starch-acclimated sludge was then used for determining the starch removal characteristics under the conditions stated above.

For studying the starch removal characteristics under unacclimated conditions, sludges were developed on glucose, maltose and bovine serum albumin substrates as described earlier. A Metrecal-acclimated sludge was also used as one of the unacclimated sludges in these studies. Starch was fed to these unacclimated sludges and its removal characteristics were determined with respect to time.

5.4.1 Acclimated Systems Under Aerobic Conditions

The acclimated activated sludge units were started from the Metrecal continuous activated sludge unit as mentioned earlier. The acclimation of the units to potato starch was determined by the attainment of equilibrium of the mixed liquor suspended solids under the feeding conditions of the unit and by the consistent rate of removal of the substrate, i.e., steady state conditions were attained. Usually, it took 7 to 10 days for the Metrecal sludge to acclimate to potato starch substrate. The daily waste sludge from the acclimation unit was removed after approximately 20 hours of aeration. The unit was in the endogenous phase after about six hours of aeration, therefore a wastage of one-third of the mixed liquor after 20 hours instead of 23 hours, did not upset the unit. The sludge collected from the acclimation unit was allowed to settle, the supernatant decanted, and the sludge homogenized in a mixer ("Omnimix") to break the clumps. An appropriate amount of sludge, depending on the particular F/M ratio desired, was taken in a glass beaker, made up to a known volume with tap water and aerated at a rate of 3000-4000 ml per minute with a glass diffuser in a constant temperature bath. Two 5-ml samples were withdrawn from the aerating sludge to determine the initial sludge concentration and the COD of the system. At a preset time, substrate potato starch (usually equivalent to 1500 mg/l COD) and nutrients consisting of ammonium chloride and phosphate buffer (pH 7.2) were added. After 30 seconds of mixing, 5 ml were withdrawn from the aerated sludge-substrate and filtered through a tared membrane filter. It usually took 30 to 60 seconds to filter this amount. Immediately after filtration, 1 ml of the filtrate was used for the starch-iodine test, and the rest was preserved in the refrigerator for the COD test (and sometimes anthrone test). The same procedure was followed

for subsequent samples at different times.

The presence of any starch-degrading exoenzyme in the medium was checked by following the starch concentration in the membrane filtrate after a certain contact period between the substrate and the microorganisms. The cell-free filtrate was incubated under the same conditions as the mixed liquor, except that there was no aeration of the filtrate, and the former was occasionally mixed in the vortex mixer. The term "cell free" used in this investigation refers to mixed liquor membrane filtrate essentially free of cells.

The pH, air flow and temperature were monitored during the run and any slight variation was corrected immediately. The three temperatures under which the potato starch removal characteristics were determined were 5°, 20° and 30°C.

The phosphate buffer was successful in keeping the pH of the units at pH 7.2, and even after utilization of all the substrate, the pH seldom dropped more than a tenth of a unit. The air flow was kept between 3000 to 4000 ml/minute by manual control of the air inlet valve to the activated sludge units. In one study, the substrate level was constant at 1500 mg/l while the sludge concentration was altered to give different F/M ratios.

In the next set of experiments, these conditions were reversed. The sludge concentration was kept constant (approximately 800 mg/l) and the substrate level was varied, all other conditions remaining the same.

5.4.2 Acclimated System with Acetate Buffer

Here, 1 M acetate buffer was used to maintain the pH of the system at 7.2 instead of phosphate buffer. The buffering system consisted of acetic acid and sodium acetate. All other conditions and experimental techniques remained the same. It should be mentioned here that since phosphate buffer was not used, the total COD fed was higher than in the phosphate-buffered system. However, the starch-COD was maintained at about 1500 mg/l.

5.4.3 Acclimated Systems Under Oxygen Deficiency

This system was similar to the acclimated system with potato starch substrate, but instead of blowing compressed air through the glass diffusers, compressed nitrogen was used. The top of the vessel used as the activated sludge unit was closed with a rubber stopper having a gas exit hole and a gas entry hole. When samples were withdrawn, the stopper was opened to allow easy access. It was necessary to close the top because the turbulence of the surface caused by the bubbling nitrogen gas could cause surface oxygenation in an open vessel.

5.4.4 Non-Acclimated Systems Under Aerobic Conditions

The sludges acclimated to Metrecal, glucose, maltose and bovine serum albumin were withdrawn from the individual acclimated activated sludge units in the same way as the sludge in acclimated systems. The treatment of the non-acclimated sludge was identical to that of the acclimated sludge at 20°C. Sampling procedures and addition of nutrients and starch substrates were also performed in a similar manner.

5.4.5 Cell Wall Isolation and Experimentation

From experimentation with the acclimated potato starch sludge system under different conditions, it became apparent that the activity of exoenzymatic starch degradation in the sludge-substrate membrane filtrate was very low. This indicated a possibility of the enzymes responsible for starch degradation (amylases) being attached to the cells. Therefore, efforts were made to isolate the cell walls of the acclimated potato starch sludge in order to test this hypothesis.

The waste sludge from the potato starch acclimation unit was allowed to settle and the supernatant wasted. The settled sludge was centrifuged at 10,000 rpm for 10 minutes and the supernatant discarded. The sludge pellet was washed by resuspending in 0.1 M phosphate buffer (pH 7.0) and centrifuging again. The resulting pellet was suspended in the phosphate buffer solution to give a cell density of approximately 3 mg/ml (dry weight). This cell preparation (about 40 ml) was introduced into the chamber of a pressure cell disintegrator, and the cells were disrupted under high pressures of about 20,000 psi (71). The disintegrated cell suspension was collected in a chilled container. The cell "mash" was centrifuged at 3000-4000 rpm for ten minutes to remove intact cells and other heavy debris (72), while the supernatant containing the cell walls and other soluble cell components was collected. Intact cells still present in the supernatant phase were removed by centrifuging the latter at 3000 to 4000 rpm. The supernatant from the second centrifugation was centrifuged at 10,000 rpm for ten minutes to separate out the cell walls from the soluble fractions (72). The supernatant from this operation containing soluble cell components was saved for later enzyme assay. The cell wall pellet was washed once with 0.1 M phosphate buffer before it was

suspended finally in fresh buffer solution. This crude cell wall preparation was examined under the microscope to check for cellular debris. This revealed some inert particles and a few intact cells appearing to be Micrococci. The number of intact cells was quite negligible in the undiluted cell wall preparation. This cell wall suspension was used to determine its starch degradation characteristics. Both the cell fractions, cell wall and soluble cell components were tested in the following manner:

- i. A known volume of the fraction in phosphate buffer was placed in a beaker.
- ii. Ammonium chloride was added in proportion to that used in the activated sludge systems.
- iii. The contents were mixed magnetically and enough tap water was added to make up the desired total volume after the addition of a predetermined quantity of potato starch solution.
- iv. The predetermined amount of starch was added.
- v. Samples were withdrawn at various intervals for starch-iodine assay (after membrane filtration if necessary).
- vi. The total protein present in the systems was also determined at periodic intervals. This would enable the calculation of the specific activity of the enzyme system.

6. EXPERIMENTAL RESULTS

The experimental results dealing with the kinetics and mechanism of starch removal in activated sludge systems operated under various conditions are presented below. The salient features of the results are reported in this chapter. A detailed discussion and an overall consideration of the problem are dealt with in the next chapter.

6.1 Acclimation of the Units

The acclimation units were started with seed sludge from the basic seed unit. They were fed the particular substrate to which acclimation was desired. The units were considered to be acclimated when there was a balance between the mixed liquor suspended solids with the daily waste and the substrate fed. This was an indication of steady state conditions. As the seed Metrecal sludge was becoming acclimated to the potato starch substrate, it was thought necessary to determine both the daily solids balance and the rate of removal of the substrate. Results and diagrams (Figures 62 through 66) for the acclimation of the units to starch, glucose, maltose, and bovine serum albumin substrates are given in the Appendix B.

6.2 Starch substrate Removal in Acclimated Systems

These experiments were performed in order to study the removal characteristics of starch under acclimated conditions with the variables discussed below in detail.

6.2.1 Constant Substrate; Variable Food-to-Microorganism Ratio (F/M), Aerobic Systems

In these experiments, the starch substrate level in the activated sludge unit was about 1500 mg/l. The acclimated mixed liquor suspended solids were altered to give a variation in the F/M ratio at the beginning of the experiment. The F/M ratios reported are based on the concentration of substrate and the sludge just at the start of the experiment without taking into account the substrate adsorbed. These experiments with starch-acclimated sludge were carried out at three different temperatures -- 5°C, 20°C, and 30°C.

6.2.1.1 Substrate Removal at 5°C

Figures 5 through 9 depict the starch removal characteristics of acclimated activated sludge under different F/M ratios at 5°C. The starch concentration has been represented by starch-COD, i.e., the concentration of starch obtained from the starch-iodine test multiplied by the appropriate factor (average value, 1.04) to convert the quantity of starch obtained to starch-COD. The total-COD represents the COD of undegraded starch at any time and the COD of degradation products. The COD of the mixed liquor prior to feeding of substrate was subtracted from the individual total-COD values to give the net COD increase due to added substrate.

It will be observed that in each case, right at the start of the experiment a part of the substrate was unaccounted for. Even extrapolation of the curve to zero time gave 120 to 360 mg/l of unaccounted total-COD at different F/M ratios. This initial uptake was attributed to the adsorption of starch by the activated sludge. There was an increase of mixed liquor suspended solids (MLSS) in almost all cases at the start corresponding to

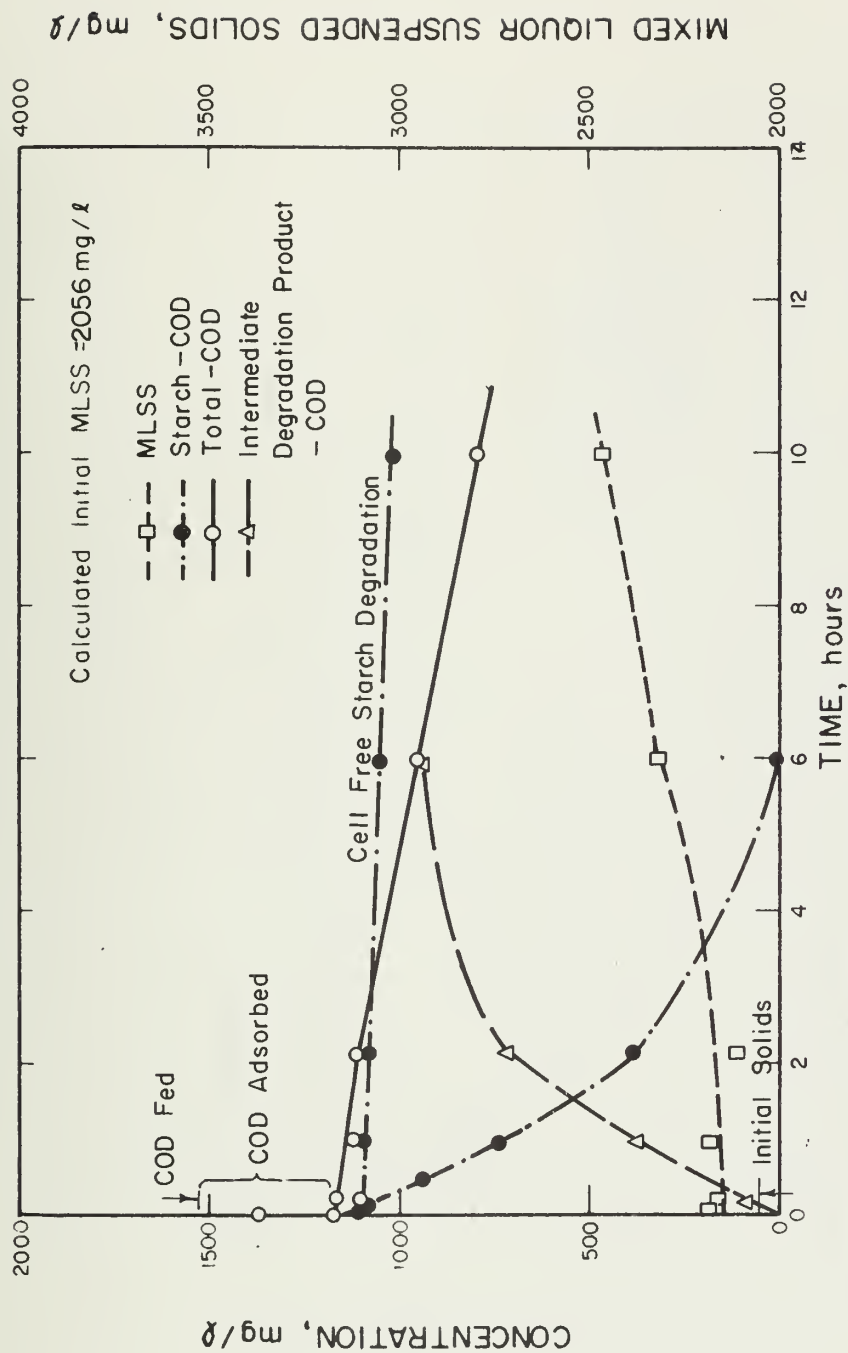


FIGURE 5 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 0.75, TEMPERATURE 5°C

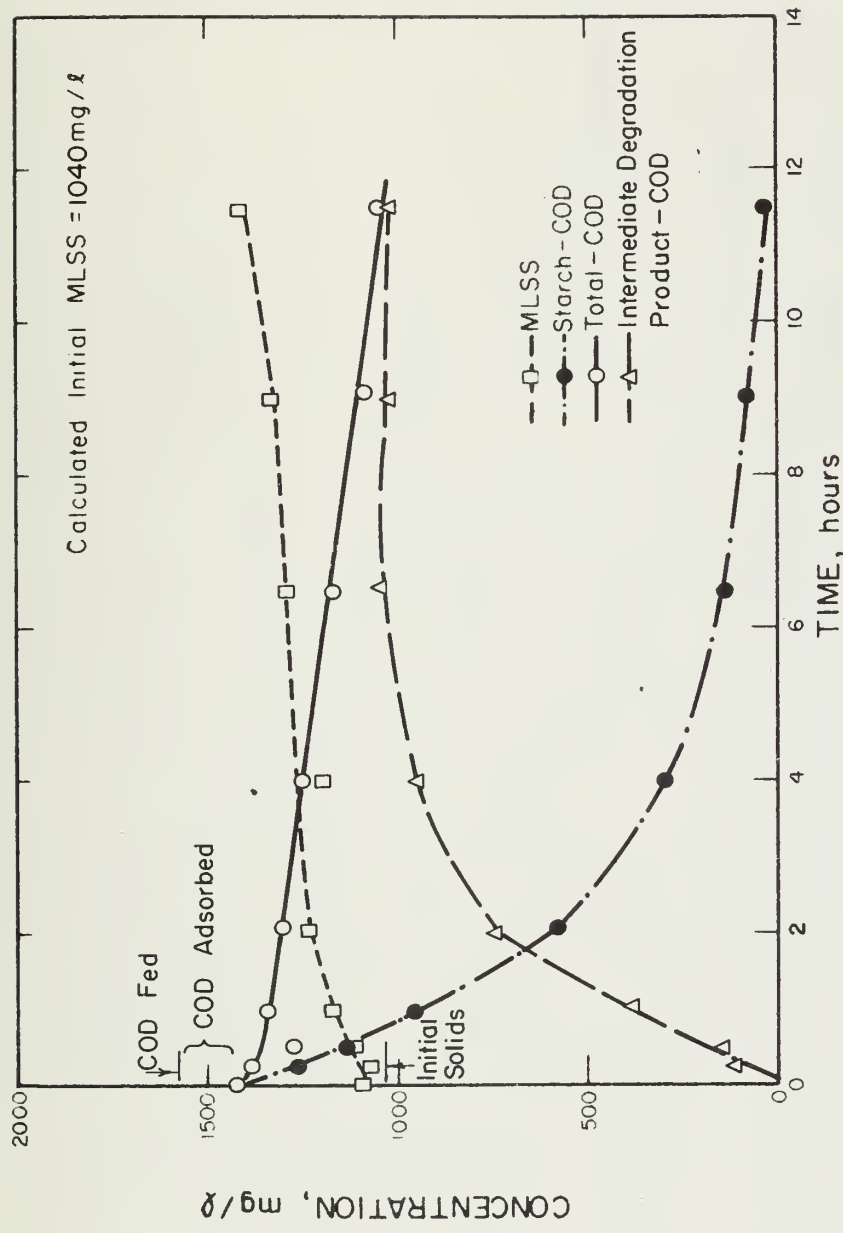


FIGURE 6 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 1.52, TEMPERATURE 5° C

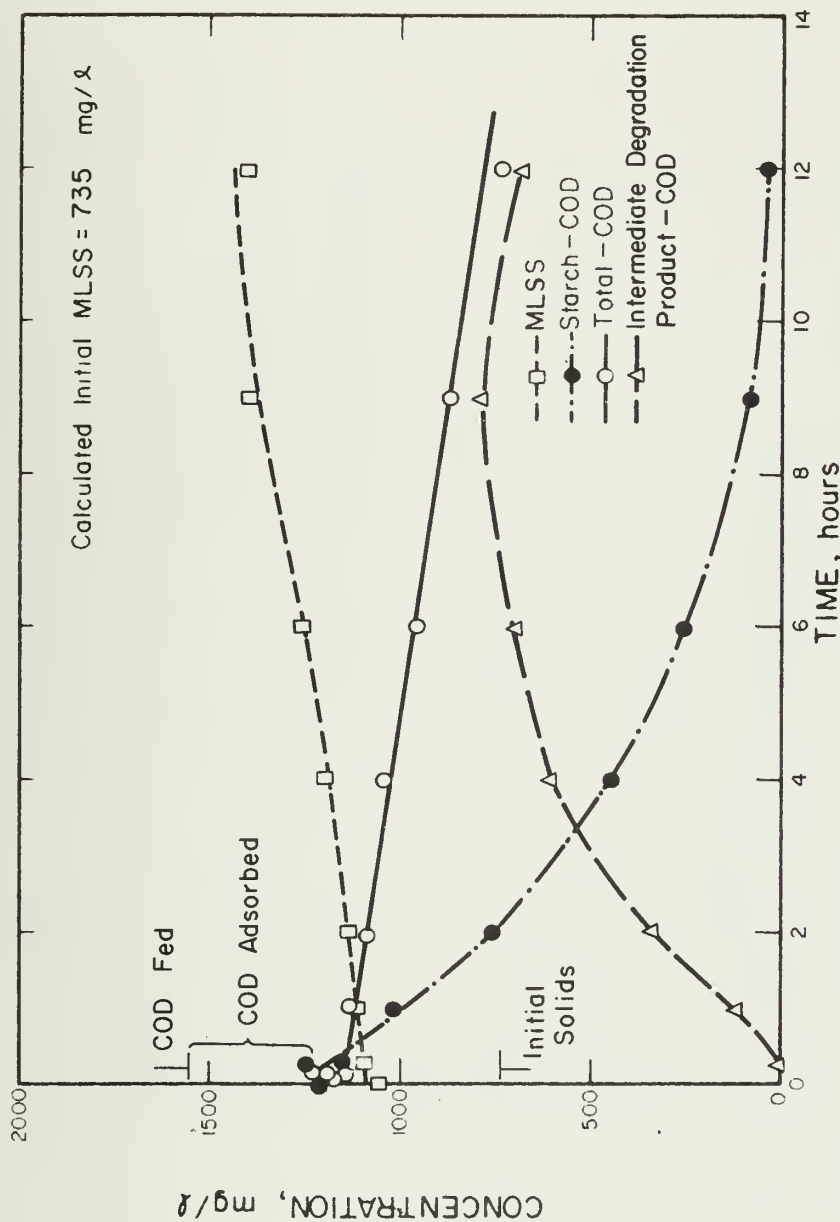


FIGURE 7 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS. F/M 2.1, TEMPERATURE 5°C

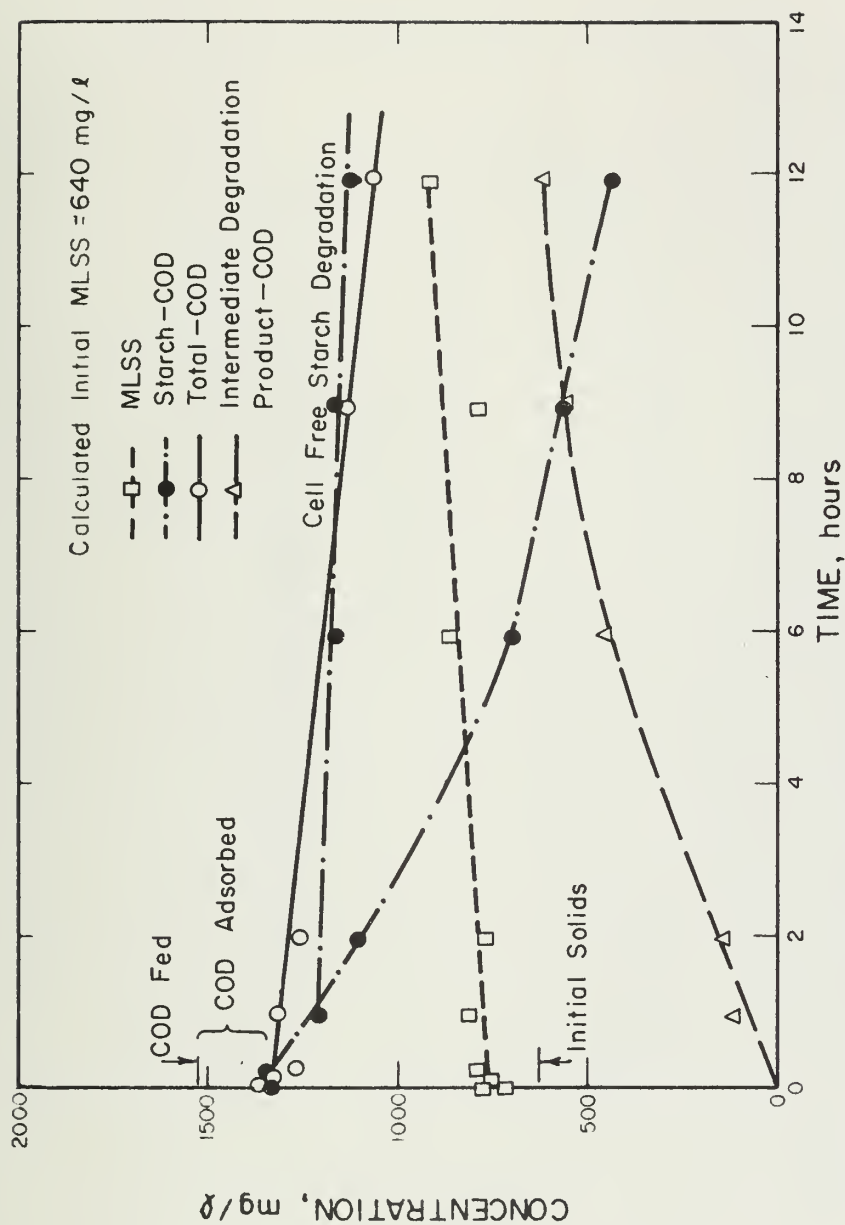


FIGURE 8 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 242, TEMPERATURE 5°C

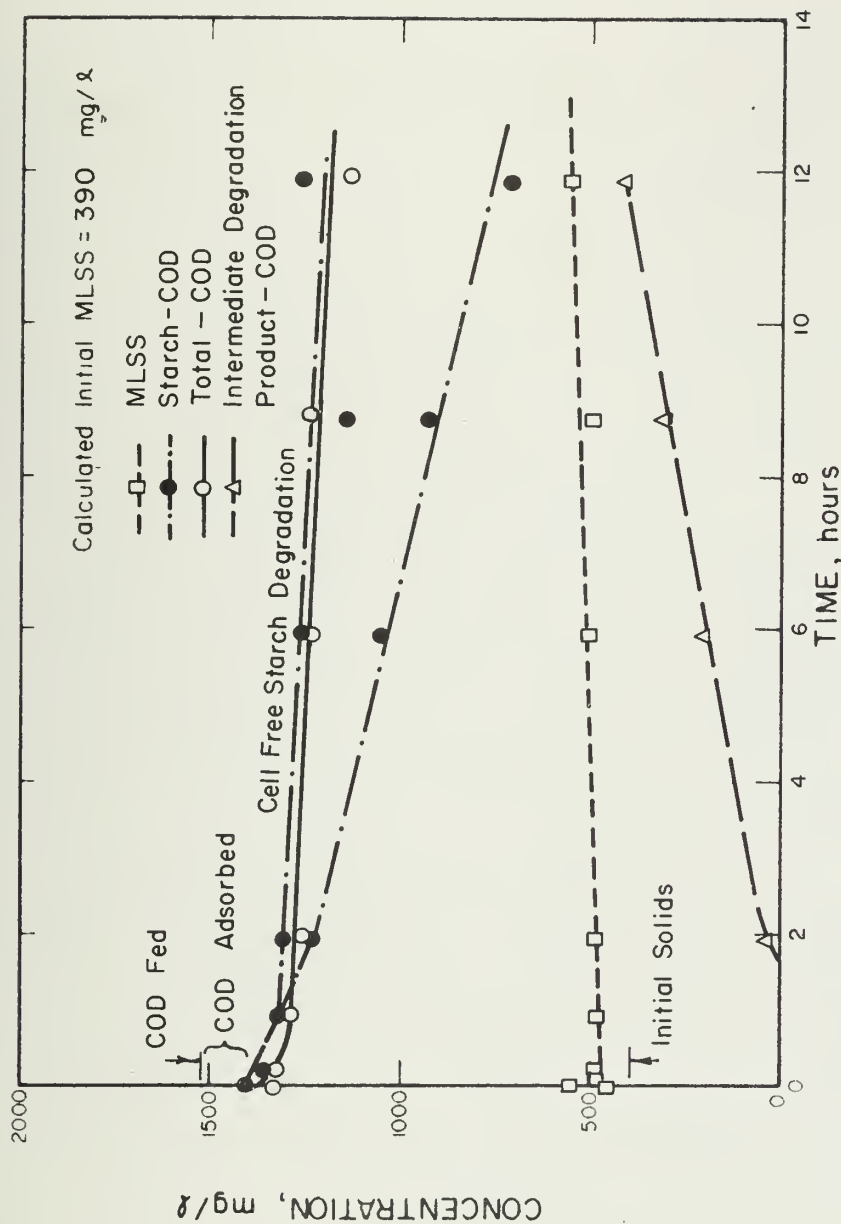


FIGURE 9 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS. F/M 3.9, TEMPERATURE 5°C

the starch adsorbed by the cells. This phenomenon of initial uptake (adsorption) has been discussed separately, for all the systems, at a later stage.

In all cases, the rate of total-COD removal was linear, although the rate of starch-COD removal was logarithmic. The starch-COD removals for the different F/M ratios were plotted on semi-log paper (Figure 10) and a linear response, at least for the first six hours, was obtained for all the systems. Table 4 summarizes the total-COD and starch-COD removal rate constants. The initial starch concentration in the filtrate at the start in each system and the initial starch adsorption are also presented. These two values add up to the amount of substrate fed. The initial total-COD adsorbed reported in Table 4 is the observed loss of substrate at the start of the experiment attributed to adsorption.

The cell-free starch-COD removal was determined on the membrane filtrate after a 10-minute contact of the cells with the substrate. The filtrate was incubated at 5°C and the starch-COD determined periodically. The cell-free starch-COD removal rate was much lower than the rate obtained with the cells. The former was linear over the period of the test as compared to the logarithmic removal rate of the latter.

The intermediate products of degradation shown in Figures 5 through 9 were calculated by subtracting starch-COD from the total-COD at a particular time. Evidently, there was a build-up of intermediate products, as the starch-COD removal was always higher than the total-COD.

6.2.1.2 Substrate Removal at 20°C

The starch removal characteristics of acclimated activated sludge with different F/M ratios at 20°C are presented in Figures 11 through 18.

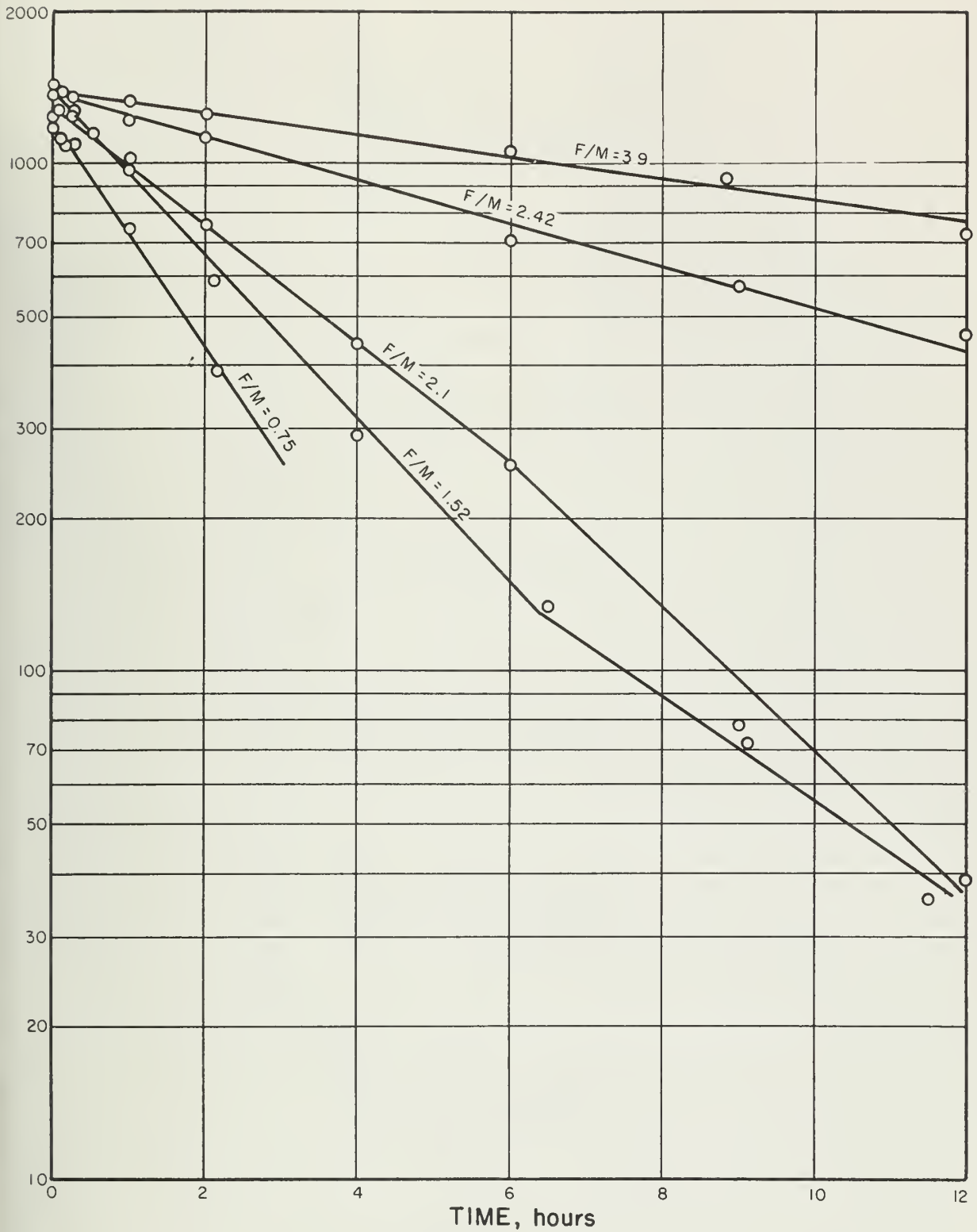


FIGURE 10 : STARCH-COD REMOVAL IN AN AEROBIC ACCLIMATED SYSTEM WITH DIFFERENT F/M RATIOS AT 5° C

TABLE 4
STARCH REMOVAL BY ACCLIMATED ACTIVATED SLUDGE SYSTEMS AT 5°C

F/M mg/mg	Total-COD Removal Rate mg/l/hr	Starch-COD Removal Rate (hr) ⁻¹	Initial Total-COD Adsorbed mg/l	Initial MLSS mg/l	Cell-Free Starch-COD Removal Rate mg/l/hr	Initial Filtrate Total-COD Concentration mg/l
0.75	40.0	0.215	360	2050	7.5	1170
1.52	29.0	0.173	152	1040	-	1434
2.1	32.5	0.112	337	735	-	1216
2.42	24.0	0.050	184	640	6.3	1360
3.9	9.0	0.025	120	390	8	1405

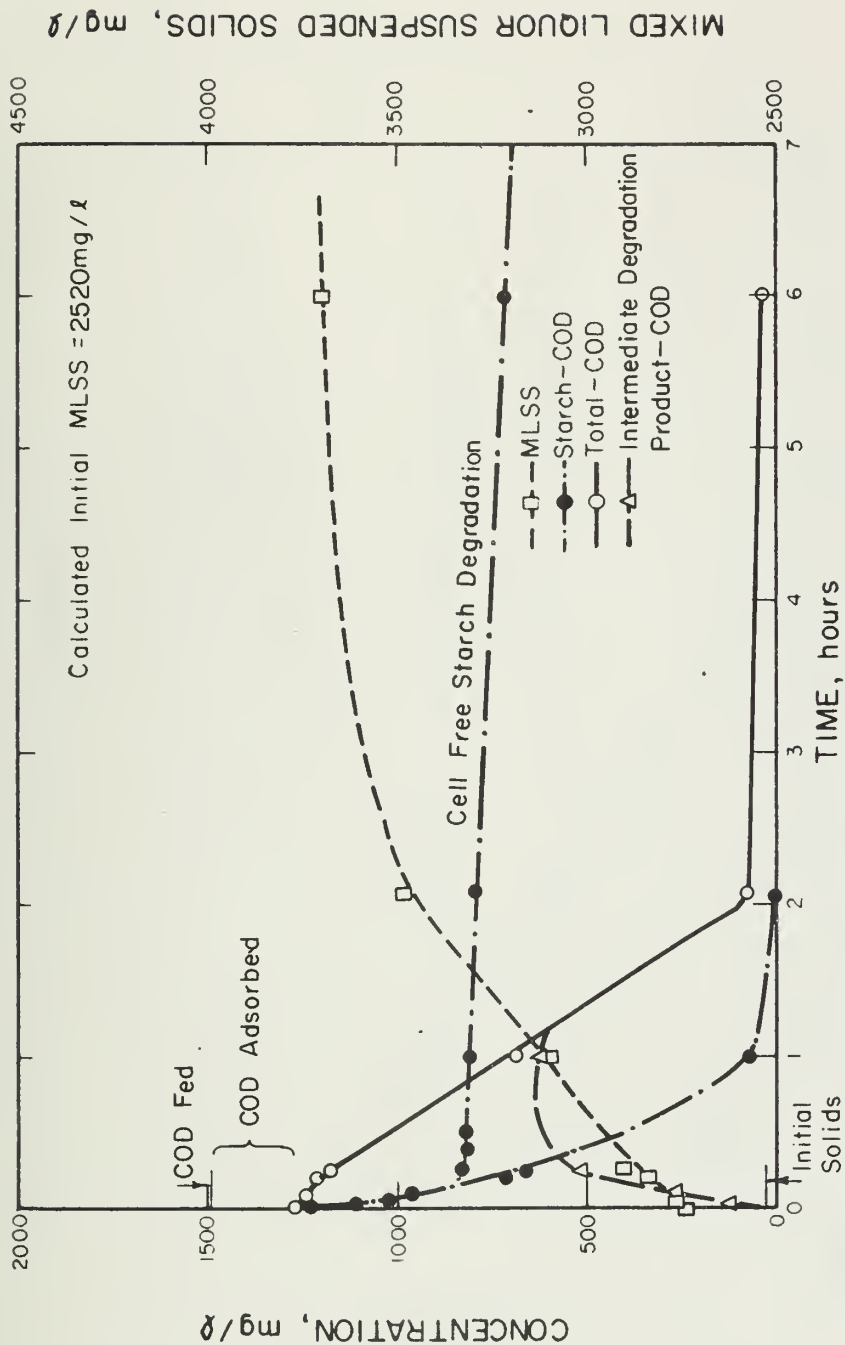


FIGURE 11 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 0.6, TEMPERATURE 20° C

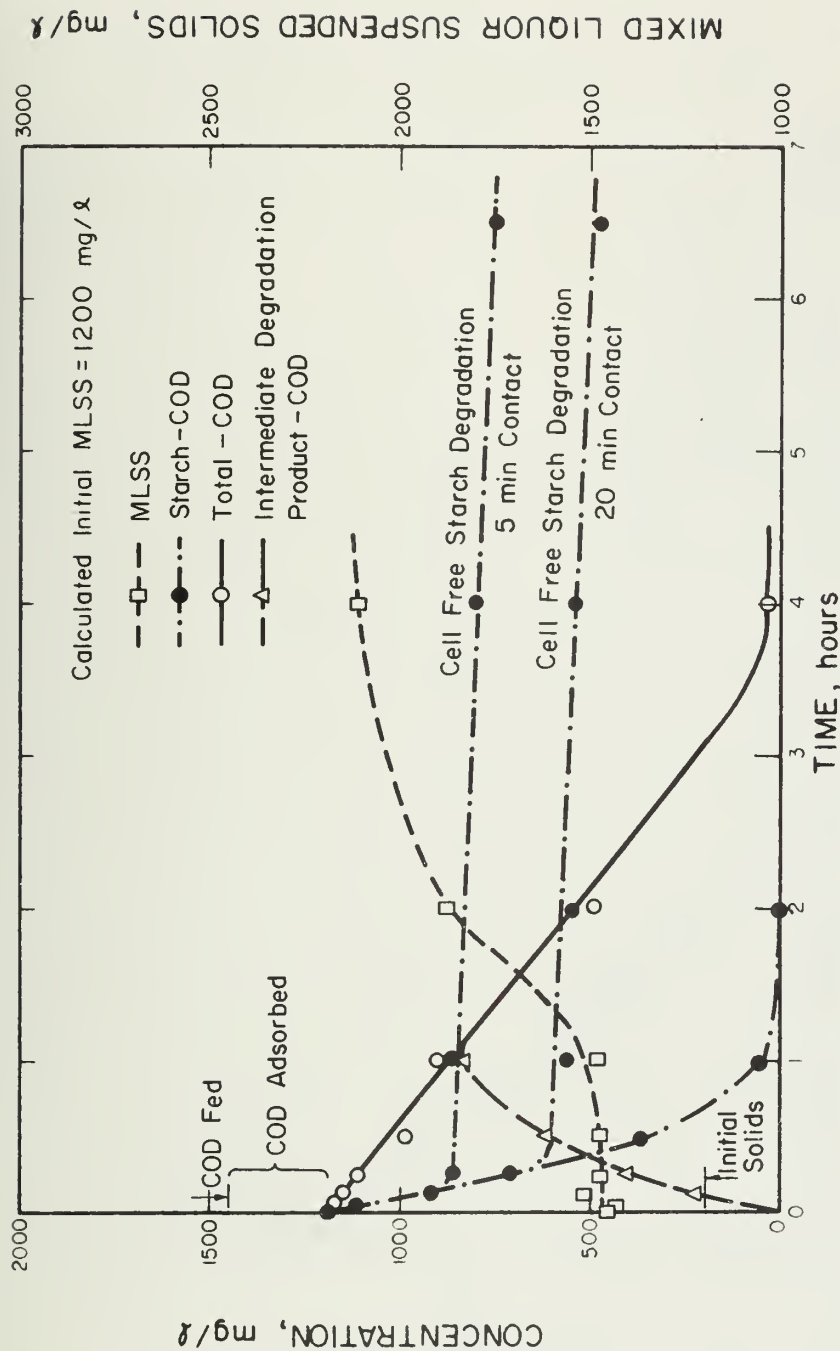


FIGURE 12 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN
ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 1.21, TEMPERATURE 20°C.

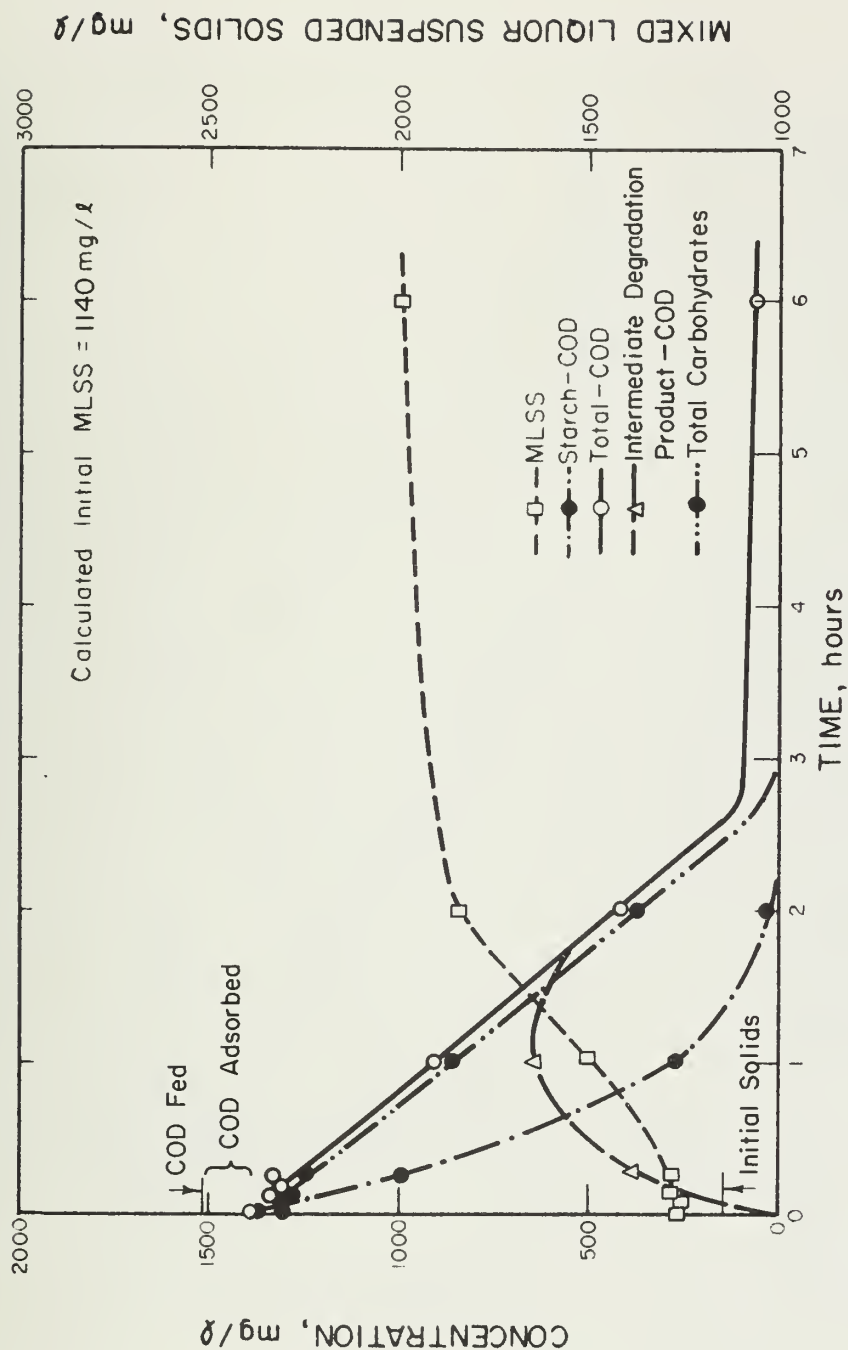


FIGURE 13 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN
ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 1.33, TEMPERATURE 20° C

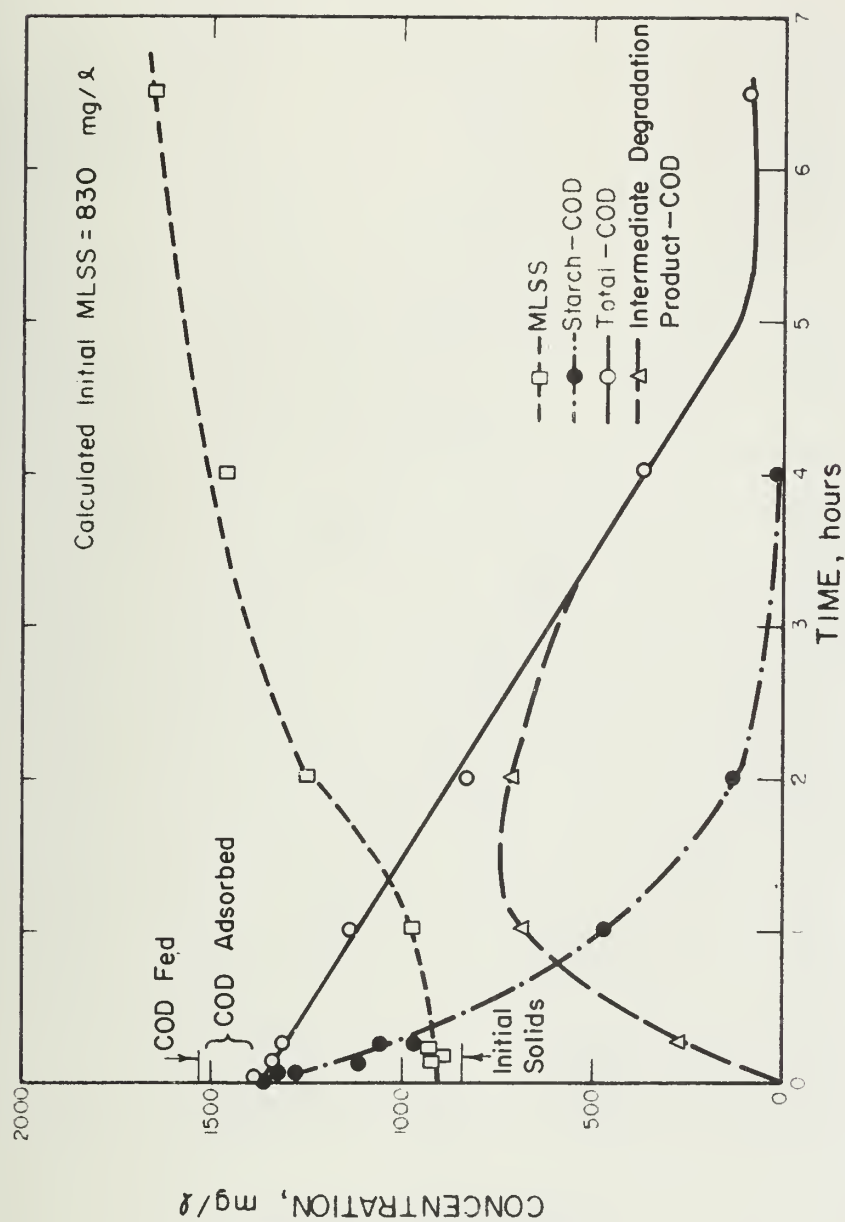


FIGURE 14 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN
ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 1.83, TEMPERATURE 20° C

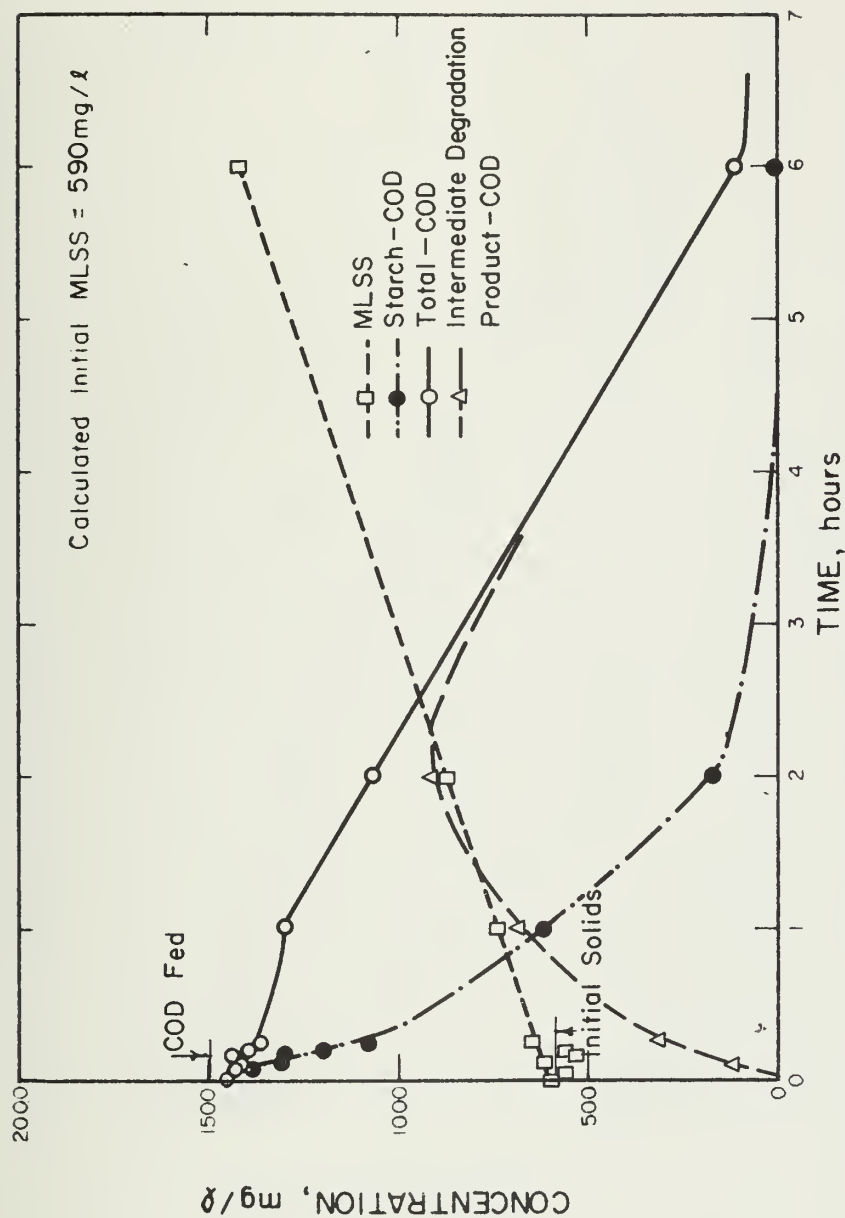


FIGURE 15 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 2.5, TEMPERATURE 20°C

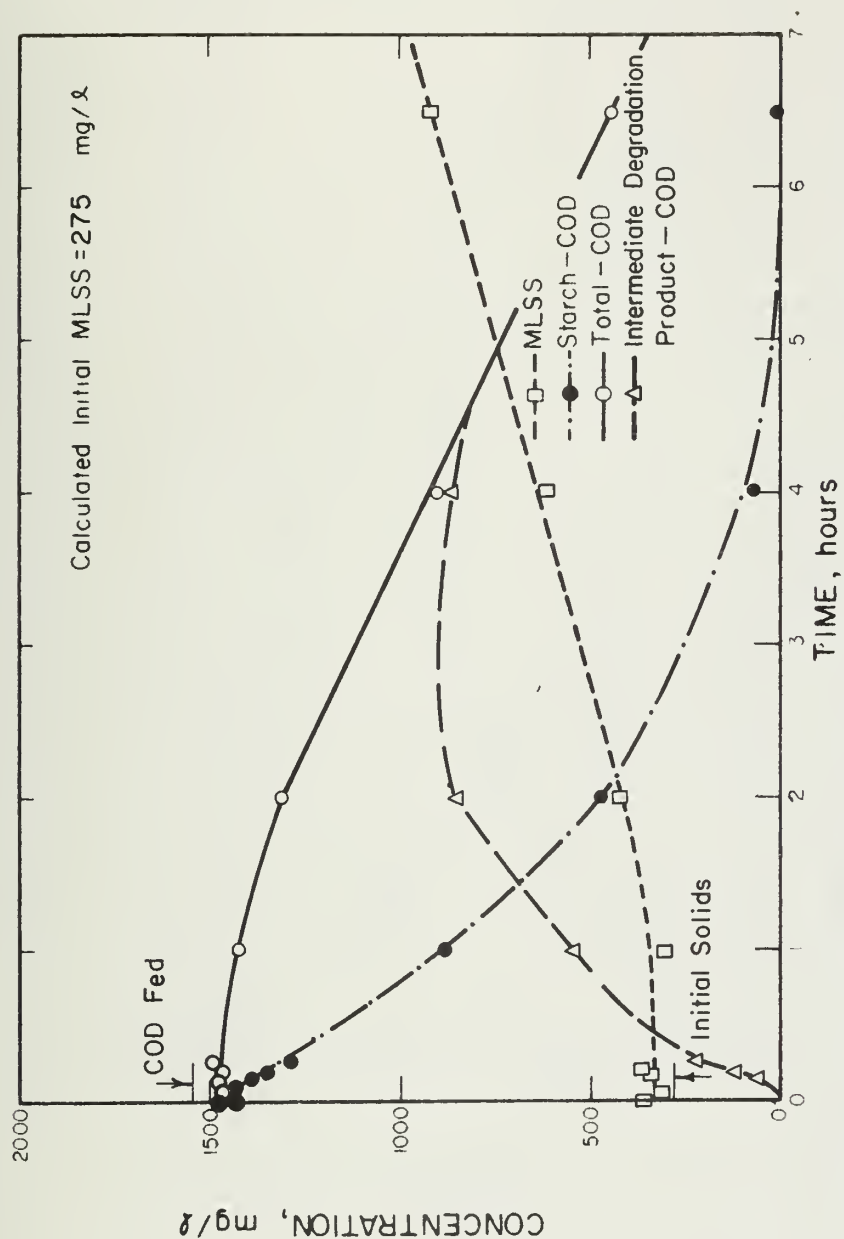


FIGURE 16 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 5.6, TEMPERATURE 20° C

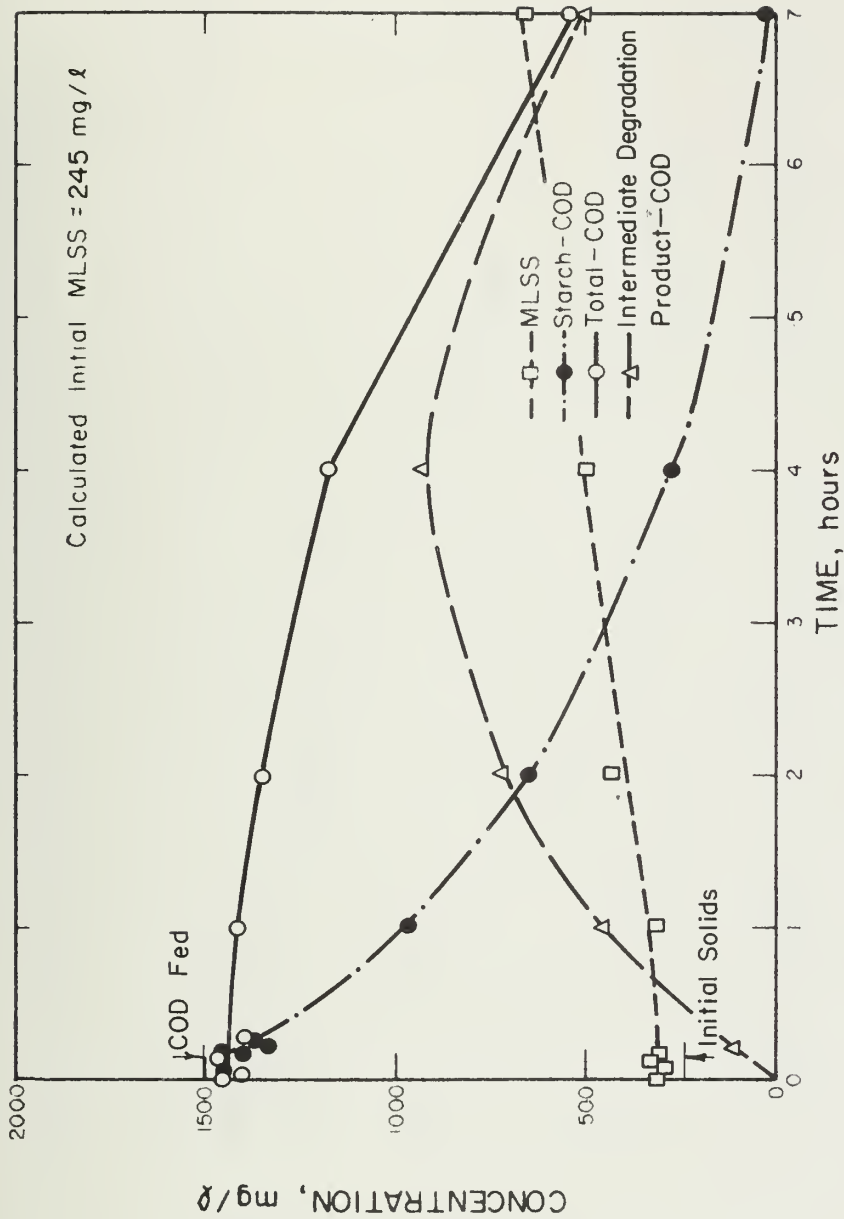


FIGURE 17 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 6.2, TEMPERATURE 20°C

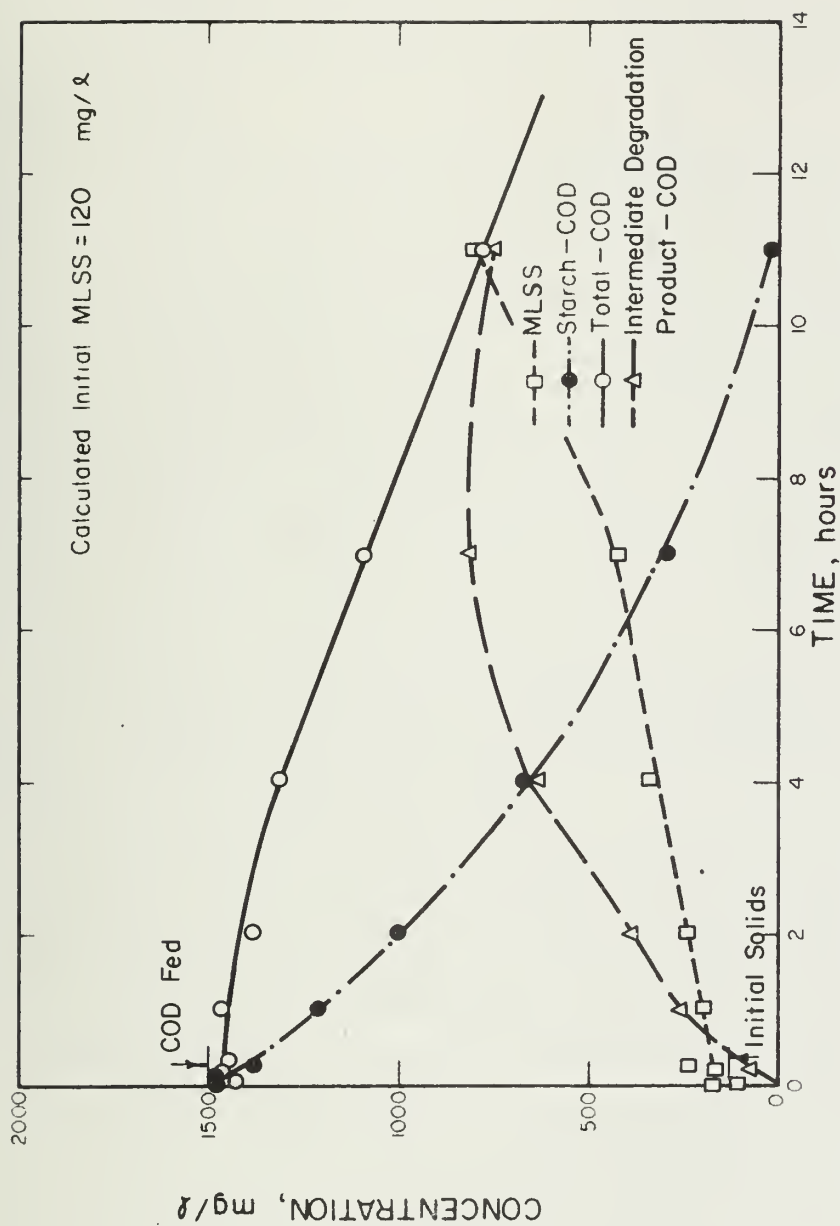


FIGURE 18 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN
ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 12.5, TEMPERATURE 20° C

Here also there was an immediate uptake of starch by the sludge at the start of the experiment which was indicated not only by the substrate loss in the filtrate but also by the increase of the initial mixed liquor suspended solids.

The total-COD removal followed a linear rate of removal after an initial lag or slower removal rate. This lag was particularly noticeable at higher F/M ratios.

The starch-COD removal rate was logarithmic for all the test runs, at least for the first two hours of aeration as indicated in Figure 19. However, in some cases there was a divergence from the logarithmic removal rate when the concentration of starch in the mixed liquor was low. The cell-free starch-COD degradation rate was linear and much lower than the starch-COD degradation in the activated sludge. This lower cell-free starch-COD degradation occurred at a lower starch concentration also (Figure 12).

The intermediate degradation products of starch are also shown in Figures 11 through 18. There was a build-up of these products till all the starch-COD in the system was removed.

Figure 20 shows the starch removal with acclimated sludge in a Warburg respirometer at 20°C and an F/M ratio of 0.92. The characteristics of total-COD and starch-COD removal were very similar to those observed with activated sludge aeration units. There was a characteristic lag initially followed by linear removal of total-COD and total-carbohydrate. A study of the total-carbohydrate curve and total-COD curve indicates that there was no accumulation of intermediate degradation products other than carbohydrate during degradation of starch in a Warburg test. This is also indicated in an acclimated activated sludge experiment (Figure 13).

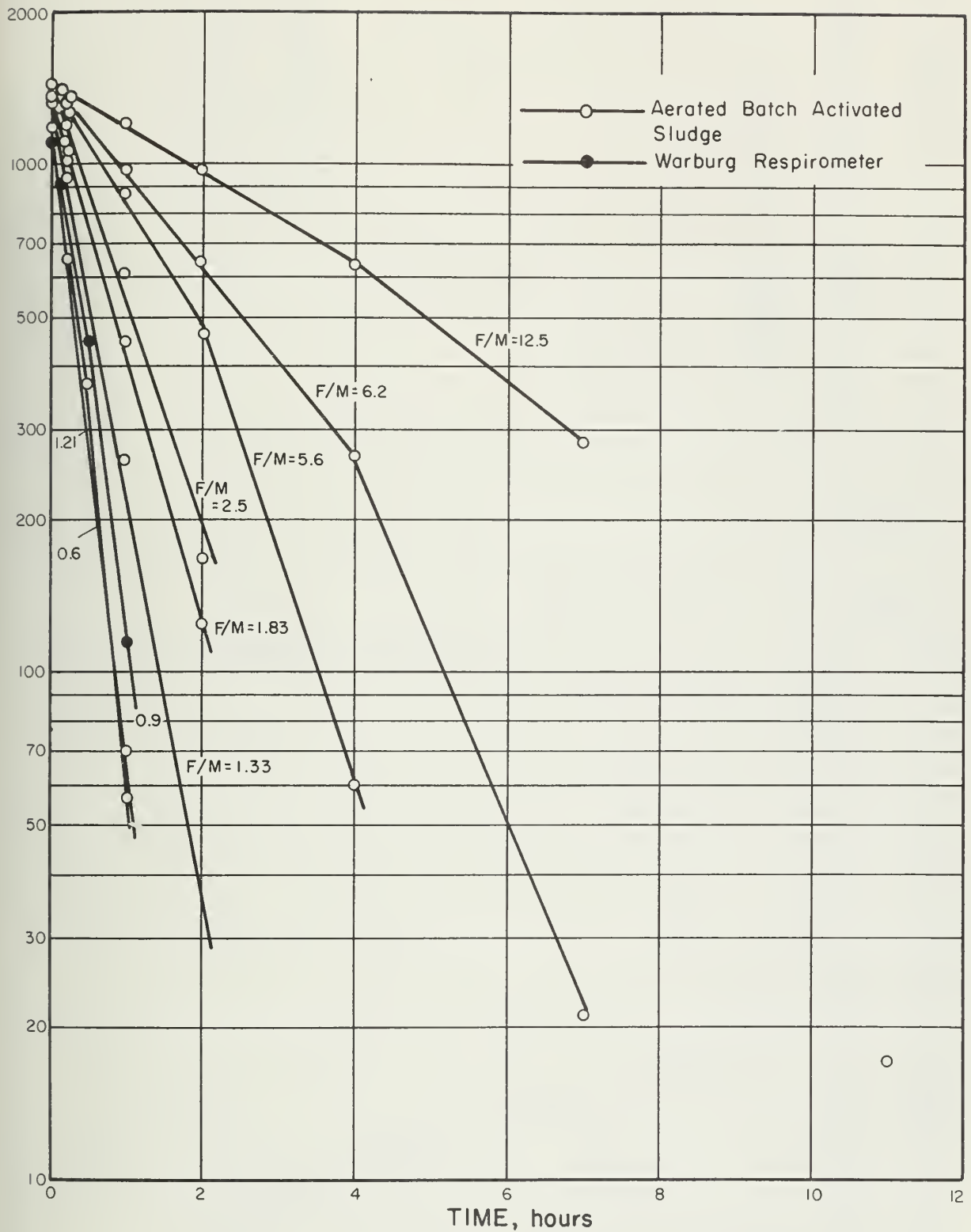


FIGURE 19 : STARCH-COD REMOVAL IN AN AEROBIC ACCLIMATED SYSTEM WITH DIFFERENT F/M RATIOS AT 20° C

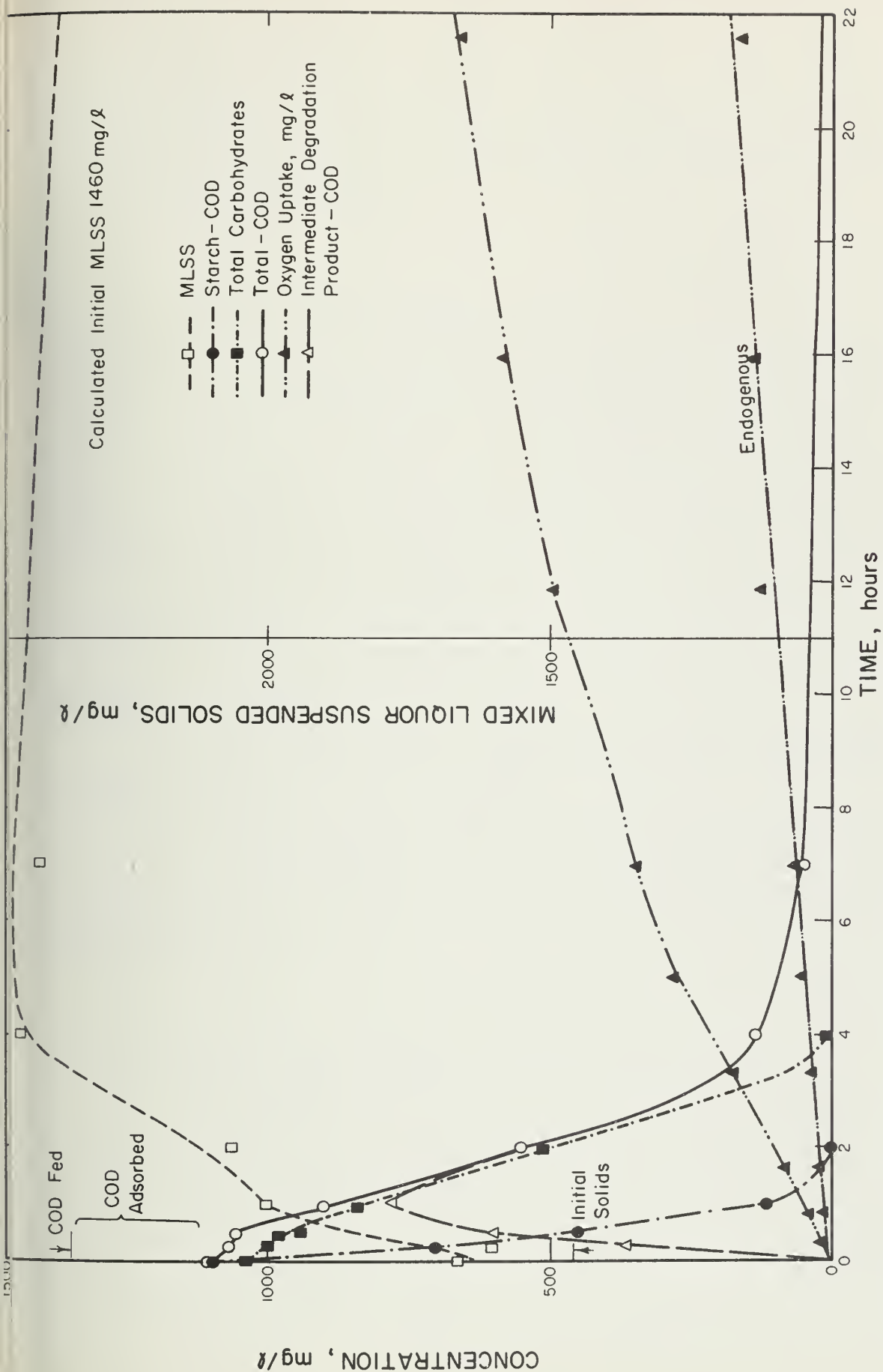


FIGURE 20: REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS IN A WARBURG RESPIROMETER. F/M 0.92, TEMPERATURE 20°C

Table 5 demonstrates the rate of total-COD removal, rate of starch-COD removal, initial adsorption, initial MLSS and initial starch concentrations. There was an increase in the rate of total- and starch-COD as the F/M was decreased.

6.2.1.3 Substrate Removal at 30°C

The characteristics of removal of starch in an acclimated activated sludge system at 30°C was very similar to that reported for 20°C, except that the removal rates for starch-COD and total-COD were higher at 30°C than at 20°C. The lag observed in the removal rate of total-COD in some cases at 20°C was quite obvious with most F/M ratios at 30°C.

Figures 21 through 26 present the data of starch removal at 30°C at different F/M ratios. The starch-COD removals were replotted in a semi-log plot in Figure 27.

The rate of total- and starch-COD removal, initial adsorption, and initial solids are reported in Table 6.

The cell-free starch degradation after a 10-minute contact period of substrate and cells in the activated sludge unit at 30°C was higher than at 20°C (Tables 5 and 6).

6.2.2 Constant MLSS; Variable F/M Ratio, Aerobic Systems

This experiment was performed in order to find out if the low activity of cell-free starch-COD degradation reported earlier was due to substrate inhibition of the enzyme systems. Further, it would be interesting to see if the rate constants (starch-COD and total-COD) were affected by variation of F/M or MLSS or both.

TABLE 5

STARCH REMOVAL BY ACCLIMATED ACTIVATED SLUDGE SYSTEMS AT 20°C

F/M	Total-COD Removal Rate	Starch-COD Removal Rate	Initial Total-COD Adsorbed	Initial MLSS	Cell-Free Starch-COD Removal Rate	Initial Filtrate Total-COD Concentration	Remarks
mg/mg	mg/l/hr	(hr) ⁻¹	mg/l	mg/l	mg/l/hr	mg/l	
0.6	630	1.255	221	2520	20	1270	Constant Substrate Concentration (Aerobic)
1.21	330	1.01	258	1200	-	1200	
1.33	480	0.796	149	1140	16	1370	
1.83	255	0.508	159	830	-	1350	
2.50	240	0.415	44	590	-	1460	
5.6	195	0.240	68	275	-	1480	
6.2	210	0.182	49	245	-	1470	
12.5	77	0.097	26	120	-	1480	Warburg (Aerobic)
0.92	340	0.778	252	1460	-	1100	
0.24	290	6.7	58	815	5	160	
0.69	228	1.32	41	750	8	470	Constant MLSS
1.52	137	0.554	210	680	17	820	(Aerobic)
1.86	235	0.450	286	820	32	1260	
1.28	-	1.320	242	670	-	1230	Oxygen Deficient (Anaerobic)
2.20	-	0.513	235	1160	-	1260	
1.4†	-	0.653	310*	930	52	1140*	Acetate Buffer pH 7.2
-	-	-	-	-	150	1500	23-hour Activated Sludge Filtrate

* Starch-COD

† Based on Starch-COD only

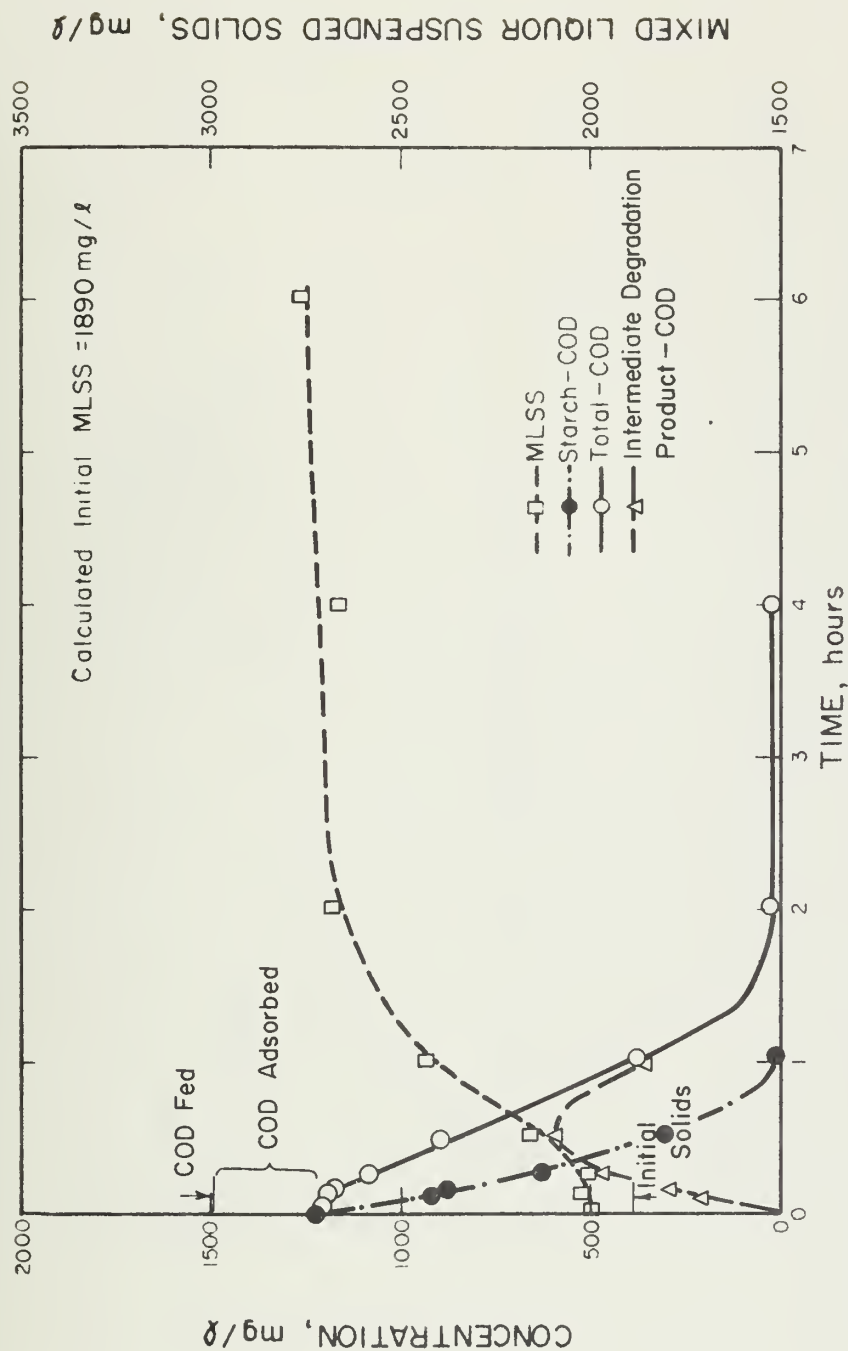


FIGURE 21 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
FM 0.79, TEMPERATURE 30° C

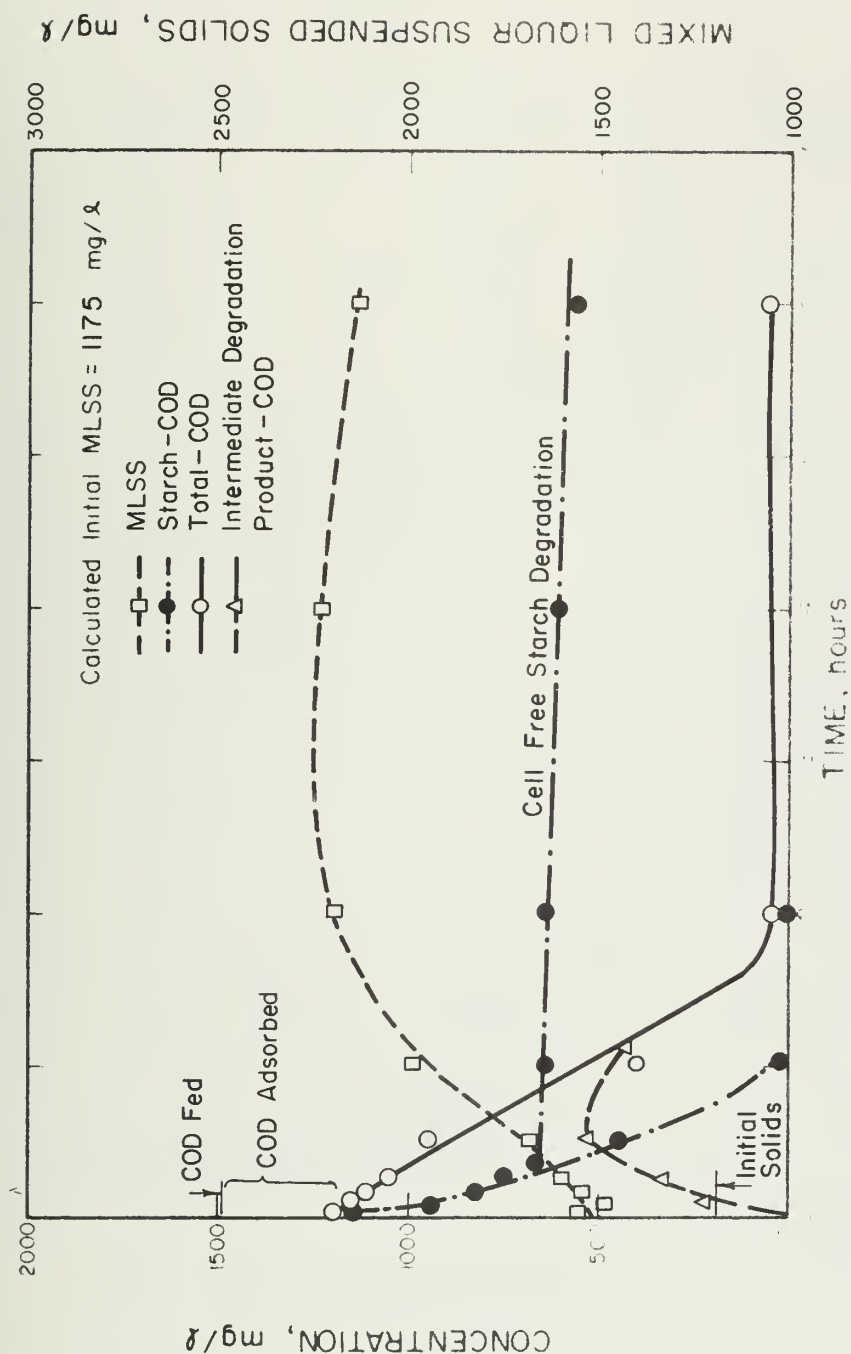


FIGURE 22 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN
ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 1.26, TEMPERATURE 30°C

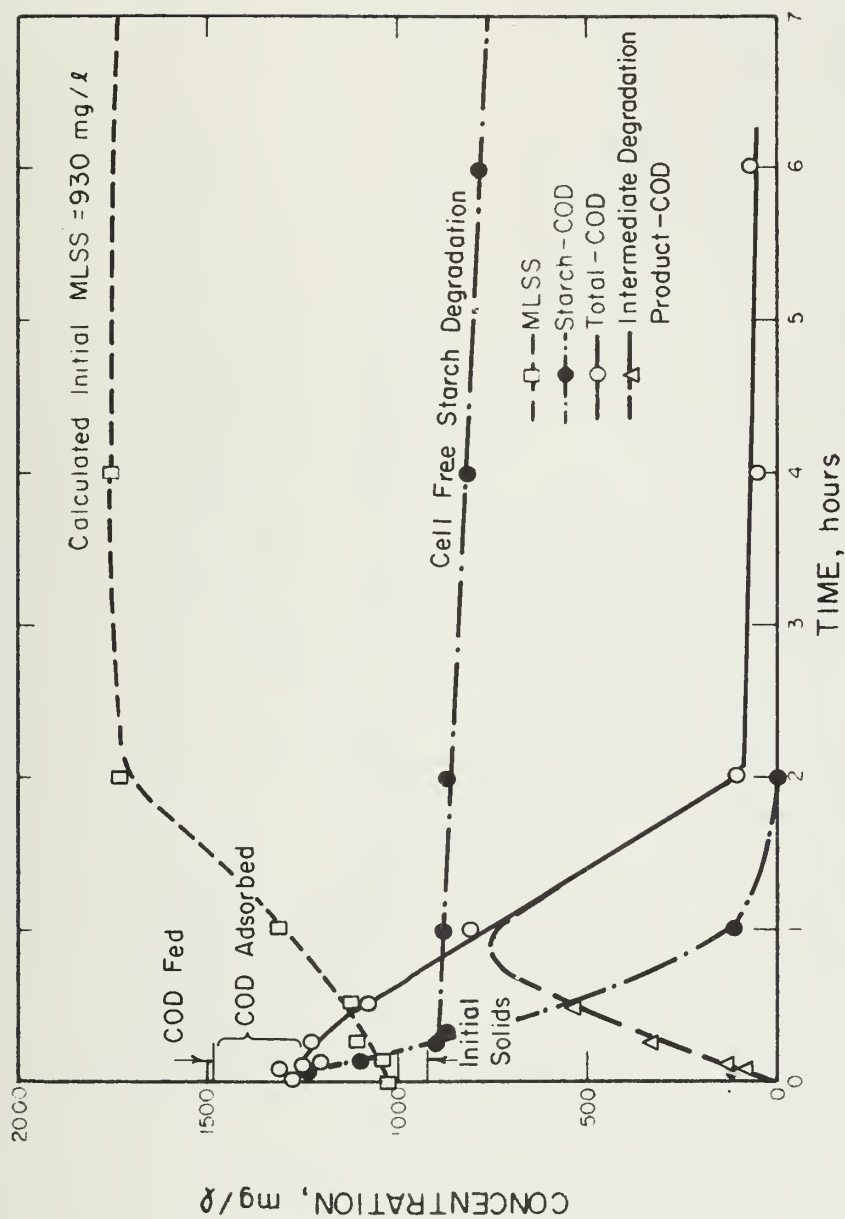


FIGURE 23 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 1.61, TEMPERATURE 30° C

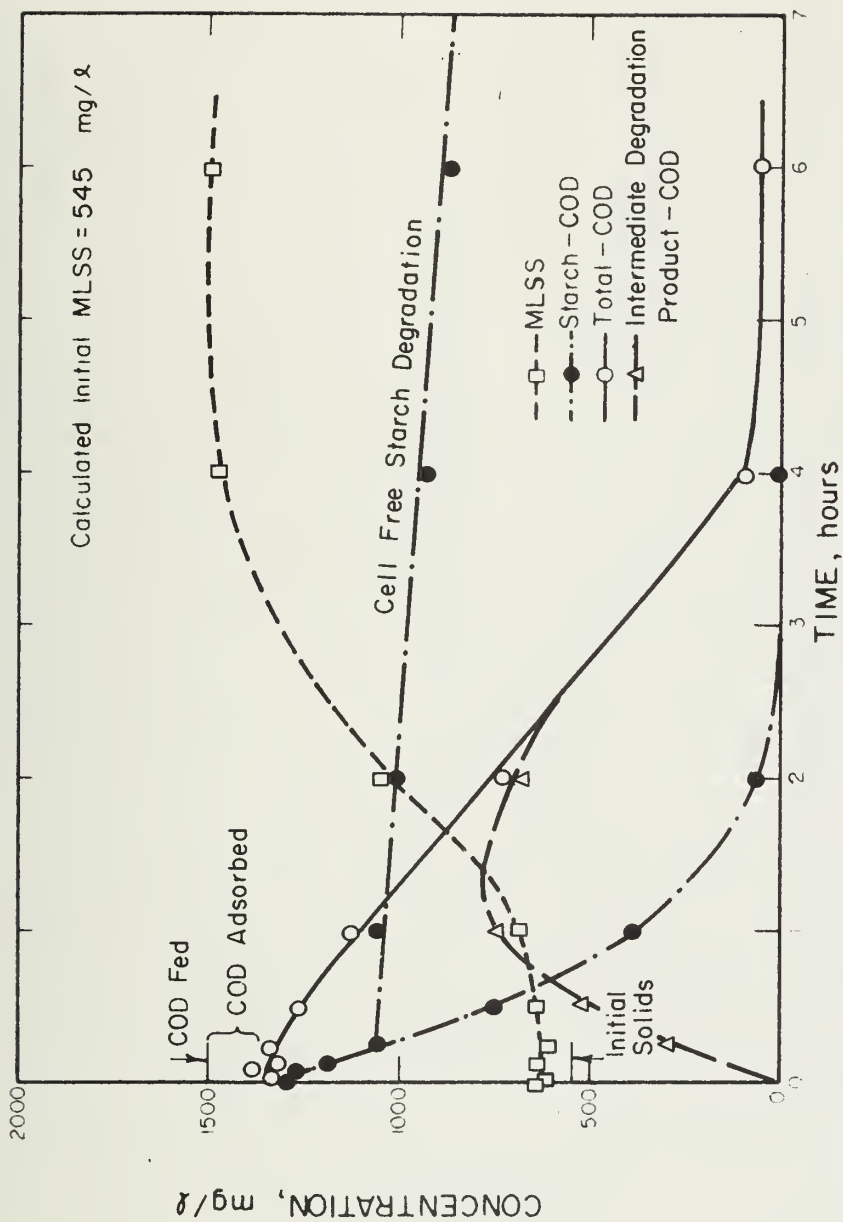


FIGURE 24 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS. F/M 2.75, TEMPERATURE 30°C

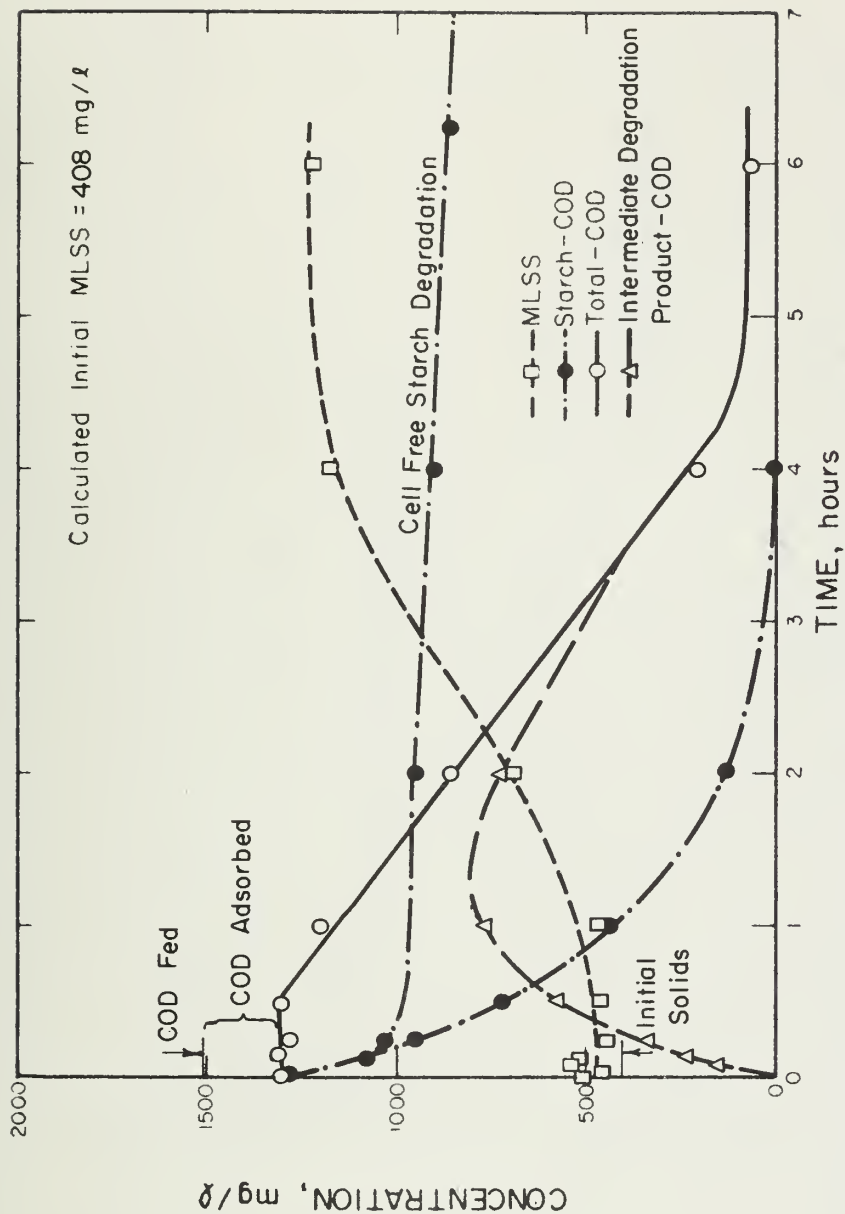


FIGURE 25 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 3.7, TEMPERATURE 30°C

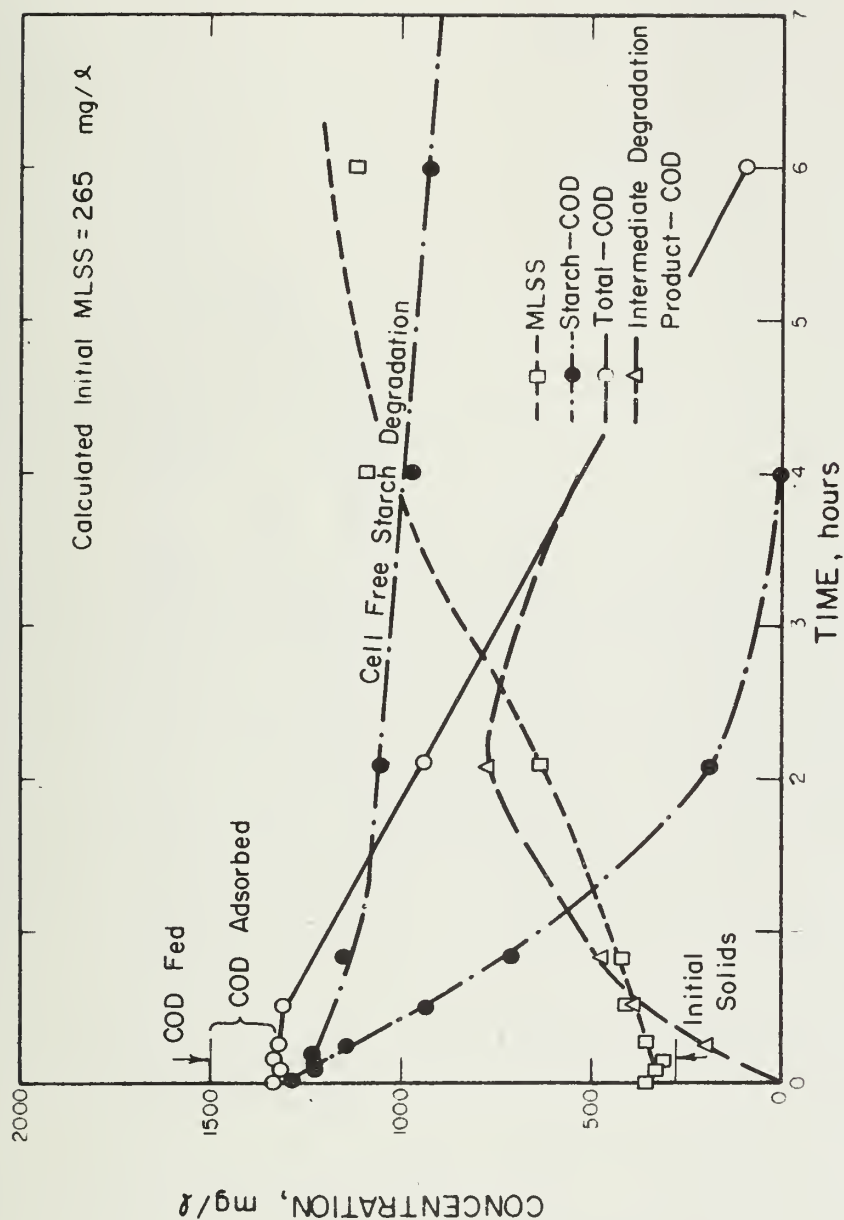


FIGURE 26 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 5.6, TEMPERATURE 30°C

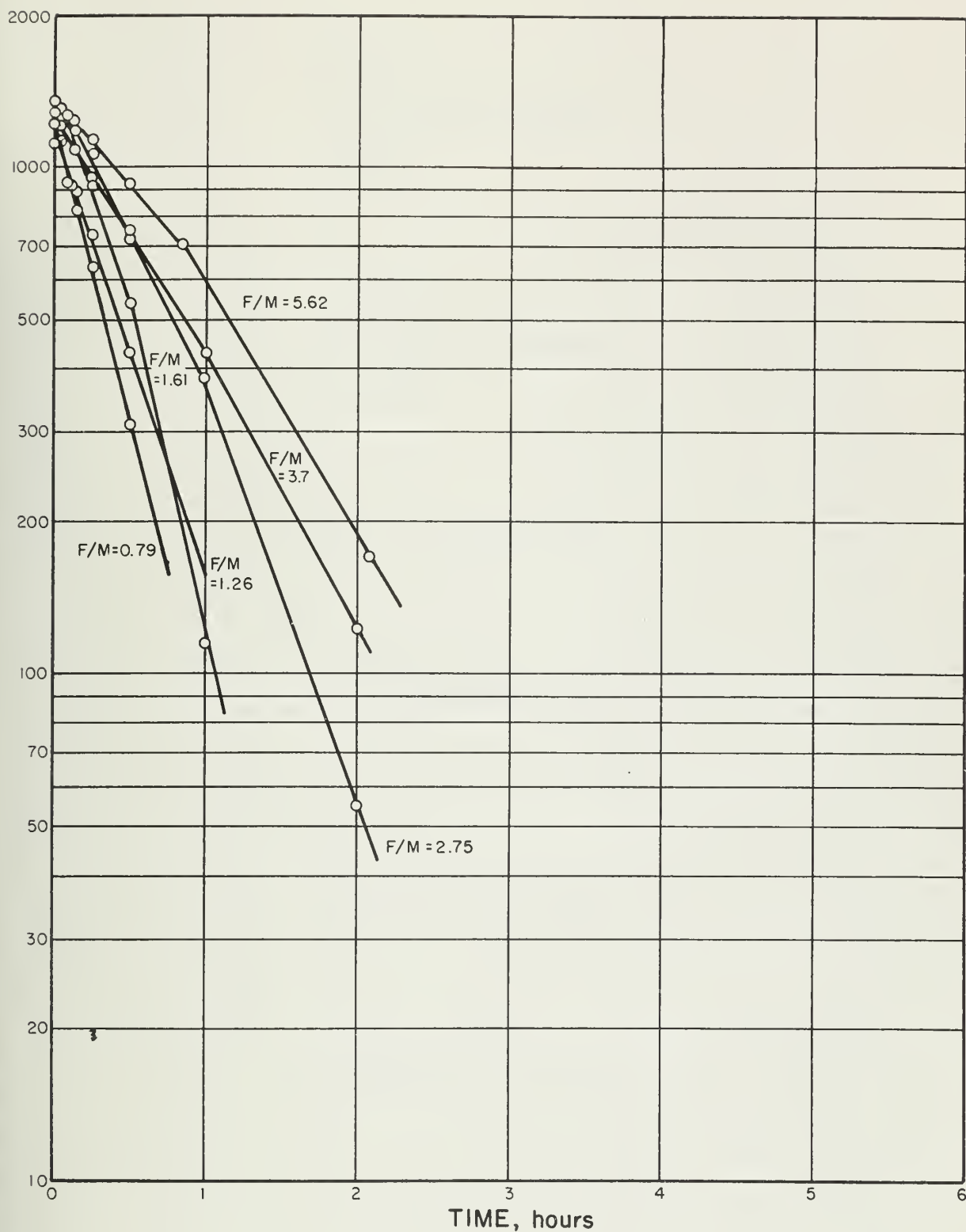


FIGURE 27 : STARCH-COD REMOVAL IN AN AEROBIC ACCLIMATED SYSTEM WITH DIFFERENT F/M RATIOS AT 30° C

TABLE 6
STARCH REMOVAL BY ACCLIMATED ACTIVATED SLUDGE SYSTEMS AT 30°C

F/M mg/mg	Total-COD Removal Rate mg/l/hr	Starch-COD Removal Rate (hr) ⁻¹	Initial Total-COD Adsorbed mg/l	Initial MLSS mg/l	Cell-Free Starch-COD Removal Rate mg/l/hr	Initial Filtrate Total-COD Concentration mg/l
0.79	940	1.192	273	1890	-	1220
1.26	690	0.84	293	1175	12	1190
1.61	640	0.75	217	930	19	1280
2.75	335	0.52	211	545	40	1350
3.7	310	0.48	207	408	22	1300
5.62	215	0.32	168	265	34	1320

In this set of experiments, the acclimated mixed liquor suspended solids were kept at about 800 mg/l, and the starch substrate concentration was varied to give different F/M ratios. These experiments were carried out at 20°C.

Figures 28 through 31 represent the starch removal characteristics at different F/M ratios but fairly constant MLSS in the acclimated activated sludge systems. The characteristics of starch removal under constant MLSS were very similar to those with constant substrate concentrations. The initial lag in the total-COD removal, especially at higher F/M values, was noticeable followed by a linear removal. The starch-COD removal rate was logarithmic (Figure 32), and the rates were higher at low F/M ratios. The cell-free starch-COD removal rate decreased as the substrate concentration decreased. Thus, the inhibition of the enzymes did not appear to be responsible for starch degradation in the cell-free system by the substrate. The starch-COD and total-COD removal rates are reported in Table 5.

6.2.3 Oxygen Deficient Systems

In this system starch was added to acclimated sludge just like the other system at 20°C, but instead of compressed air diffusion through the diffusers, compressed nitrogen was used.

Figures 33 and 34 show the starch-COD removal with a nitrogen-blown, anaerobic activated sludge system. The starch-COD was removed in a manner similar to that reported earlier with aerobic conditions (Figure 32). The MLSS and total-COD of the system were practically unchanged during the experiment. However, there was a slight decrease in the total-carbohydrates after six hours of nitrogen diffusion. Apparently, the enzymes responsible

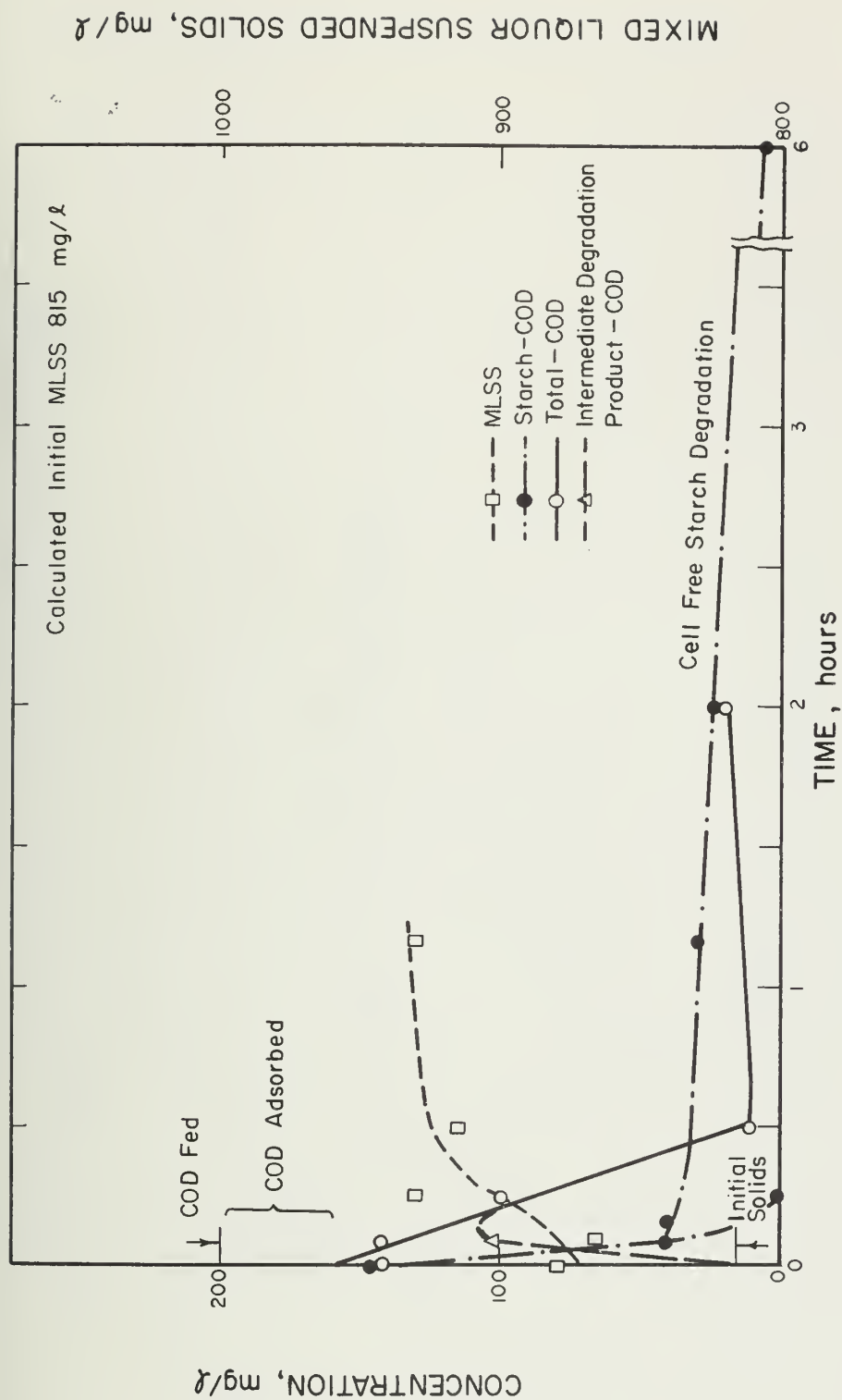


FIGURE 28 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS WITH CONSTANT MLSS. F/M 0.24, TEMPERATURE 20°C

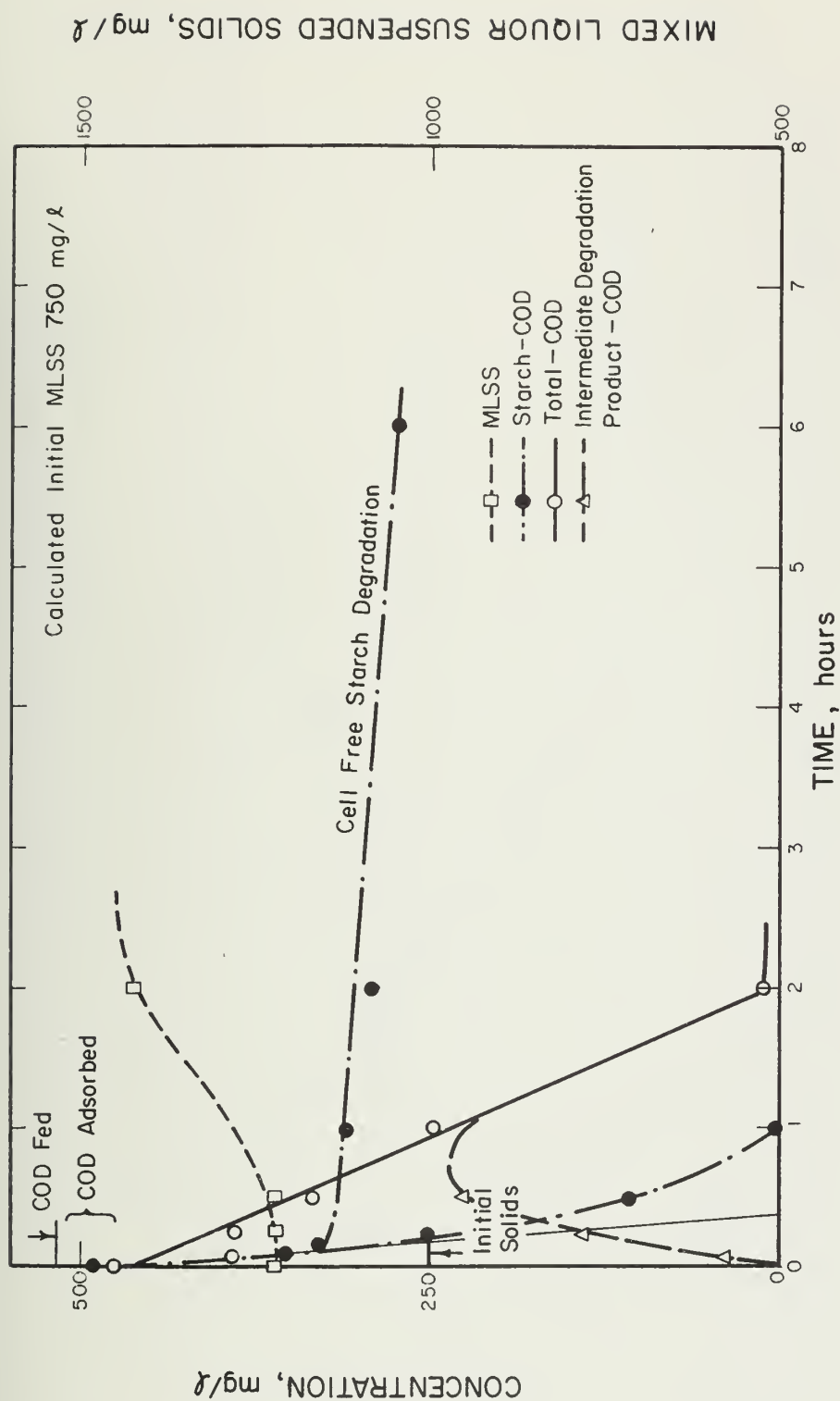


FIGURE 29: REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS WITH CONSTANT MLSS. F/M 0.69, TEMPERATURE 20° C

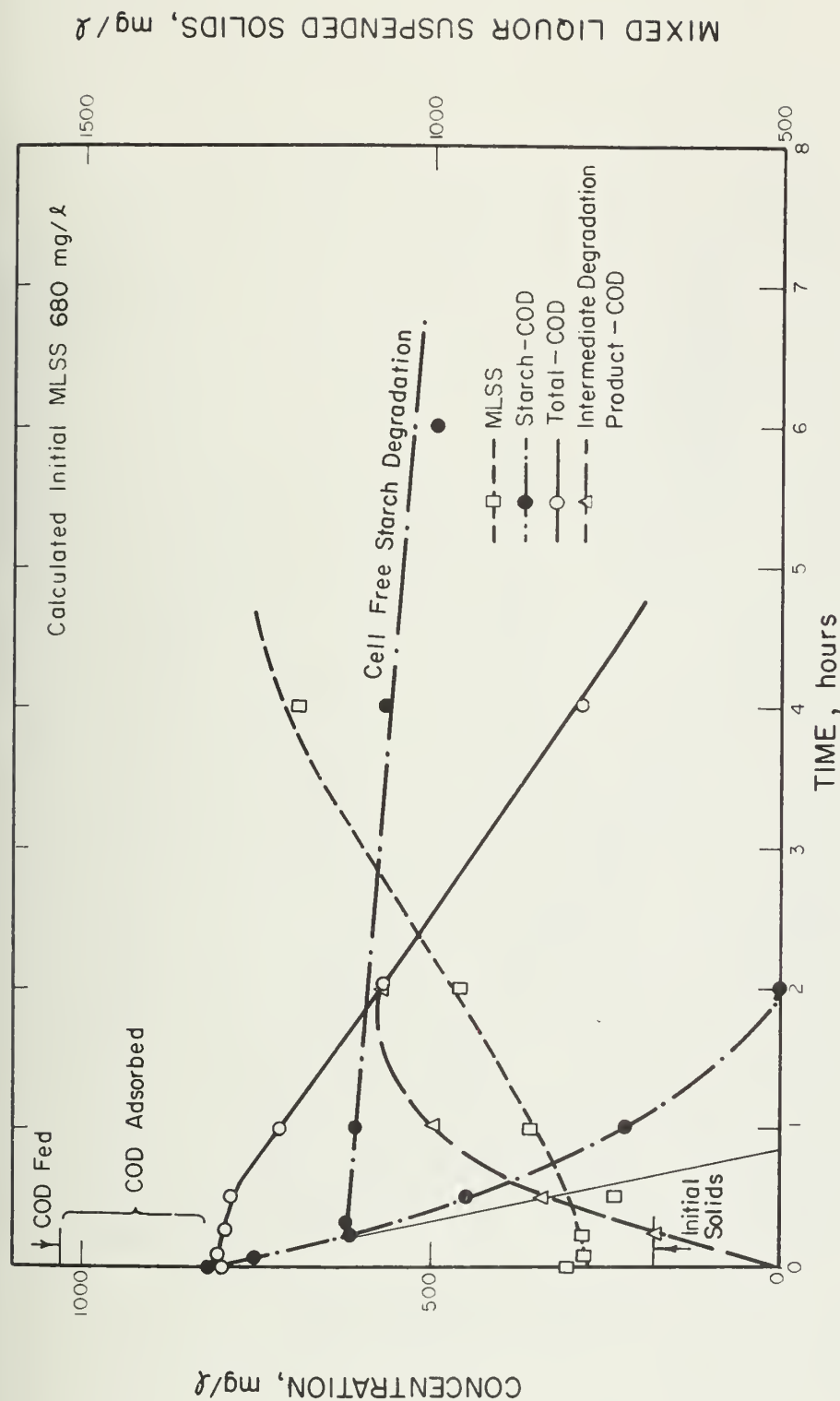


FIGURE 30: REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS WITH CONSTANT MLSS. F/M 1.52, TEMPERATURE 20° C

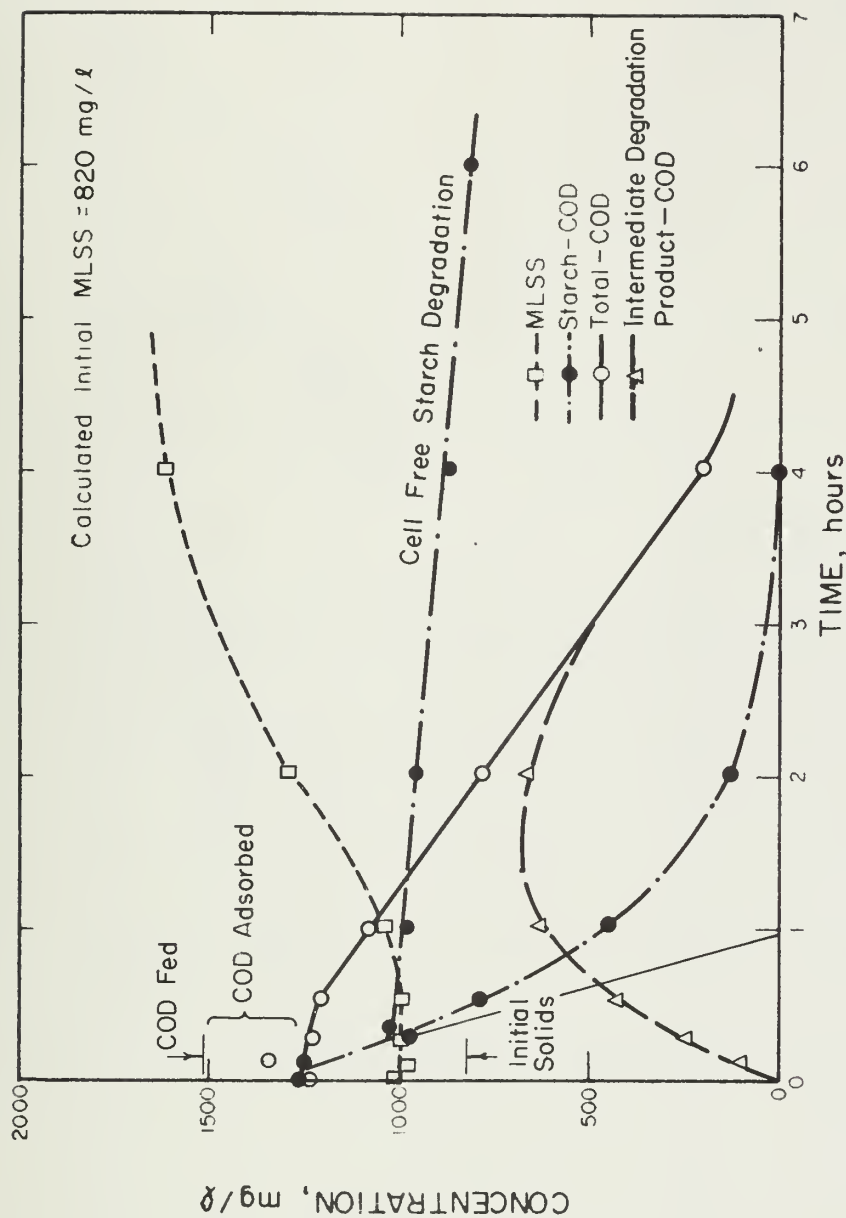


FIGURE 31 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS WITH CONSTANT MLSS. F/M 1.86, TEMPERATURE 20°C

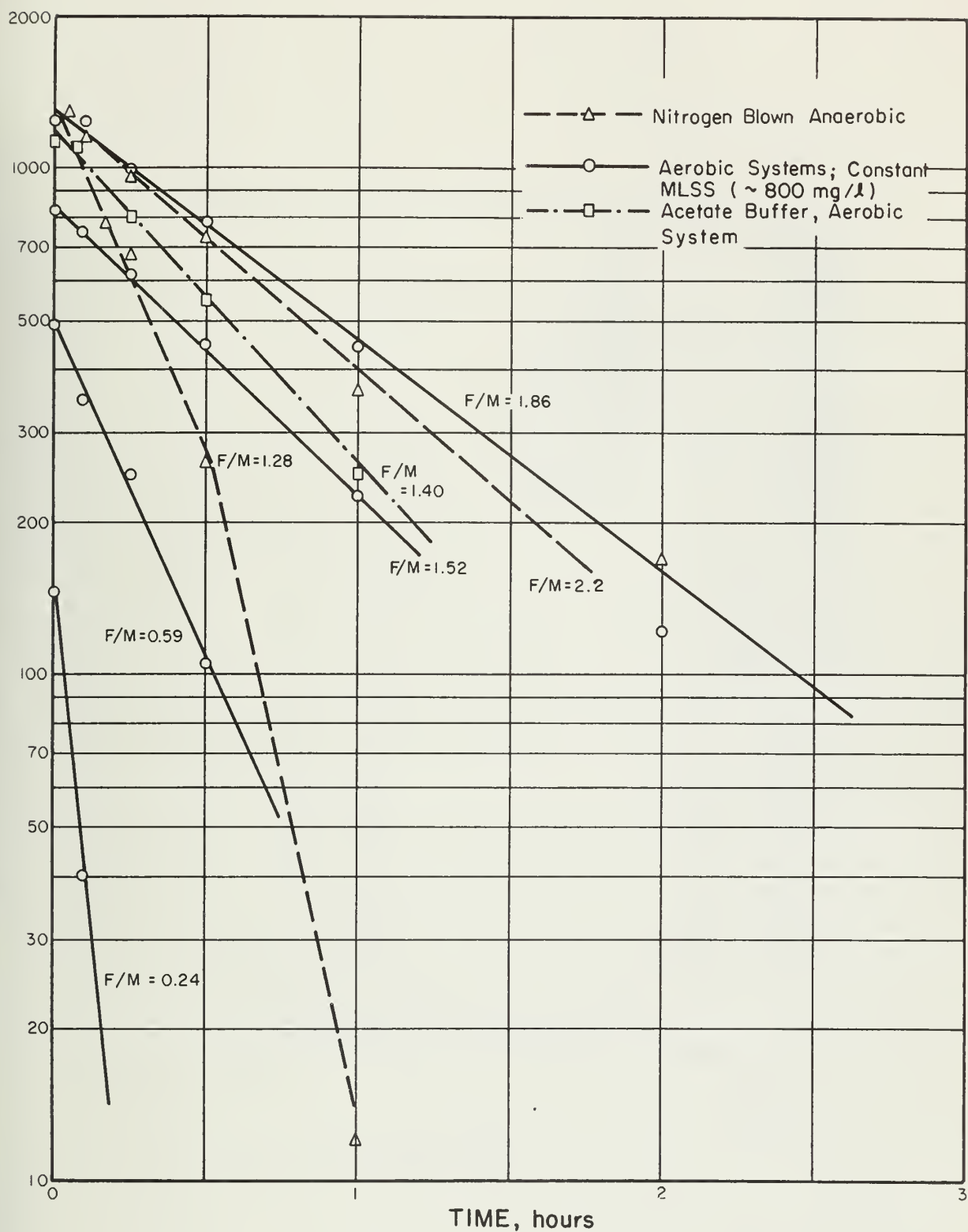


FIGURE 32 : STARCH-COD REMOVAL IN AN ACCLIMATED SYSTEM UNDER VARIOUS CONDITIONS, AT 20° C

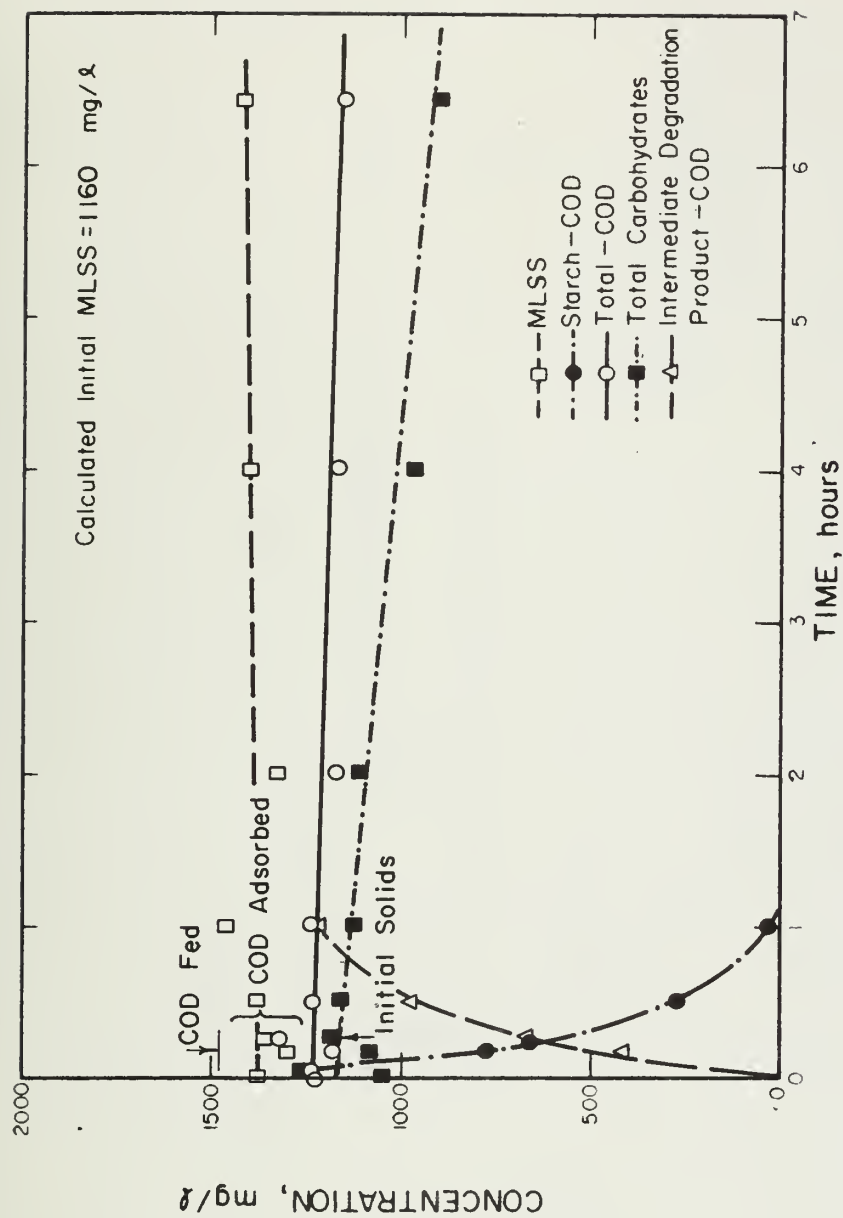


FIGURE 33 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER ANAEROBIC CONDITIONS. F/M 1.28, TEMPERATURE 20°C

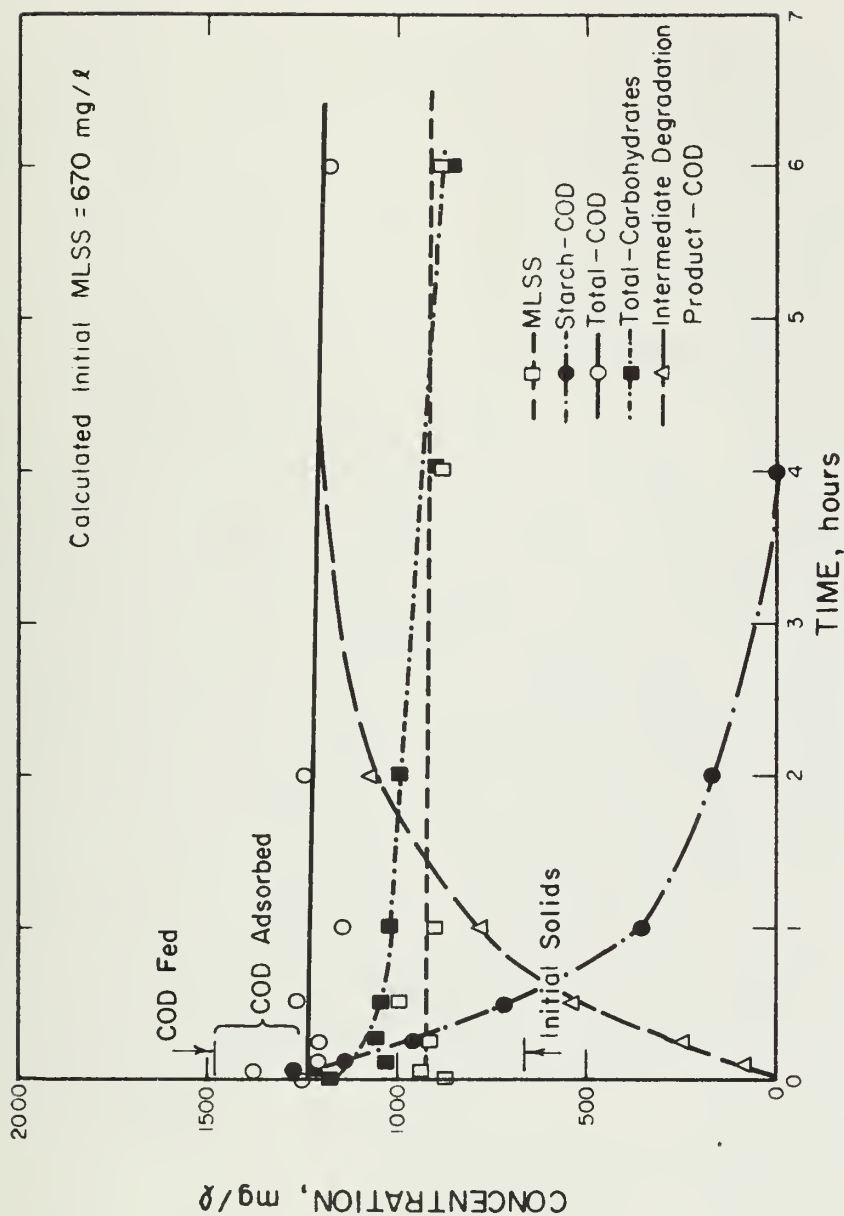


FIGURE 34: REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM UNDER ANAEROBIC CONDITIONS.
F/M 2.2, TEMPERATURE 20°C

for the degradation of starch were active even under anaerobic conditions. An initial starch adsorption on the sludge occurred as it had under aerobic conditions. Table 5 also reports the starch-COD removal rate and the initial starch adsorption under oxygen deficient systems.

6.2.4 Constant Substrate; Acetate Buffer Aerobic System

The low activity of cell-free starch degradation with acclimated sludge has been reported earlier. In all of these experiments phosphate buffer was used. It has been reported (73) that calcium ions are needed to activate amylases of some organisms and phosphate buffer was detrimental to the amylases of such systems. Therefore, the possibility existed that because of removal of calcium by phosphate buffer, the cell-free starch degradation was low. Acetate buffer (pH 7.2) was used in an experiment at 20°C with acclimated activated sludge and the starch-COD fed was 1445 mg/l, although the total-COD was much higher (about 3000 mg/l) because of the acetate-buffer added. Figure 35 represents the starch-COD degradation of aerobic acclimated activated sludge at 20°C with an acetate buffer system. It will be evident that starch-COD removal was very similar to that obtained with phosphate buffer earlier (Table 5). The cell-free starch-COD removal rate was higher than that obtained under similar conditions with phosphate buffer but was still much lower than that obtained with whole-cell systems.

6.2.5 Cell-Free Starch Degradation

Tables 4, 5 and 6 have reported the rate of cell-free starch-COD degradation with acclimated sludges at 5°, 20° and 30°C. It will be

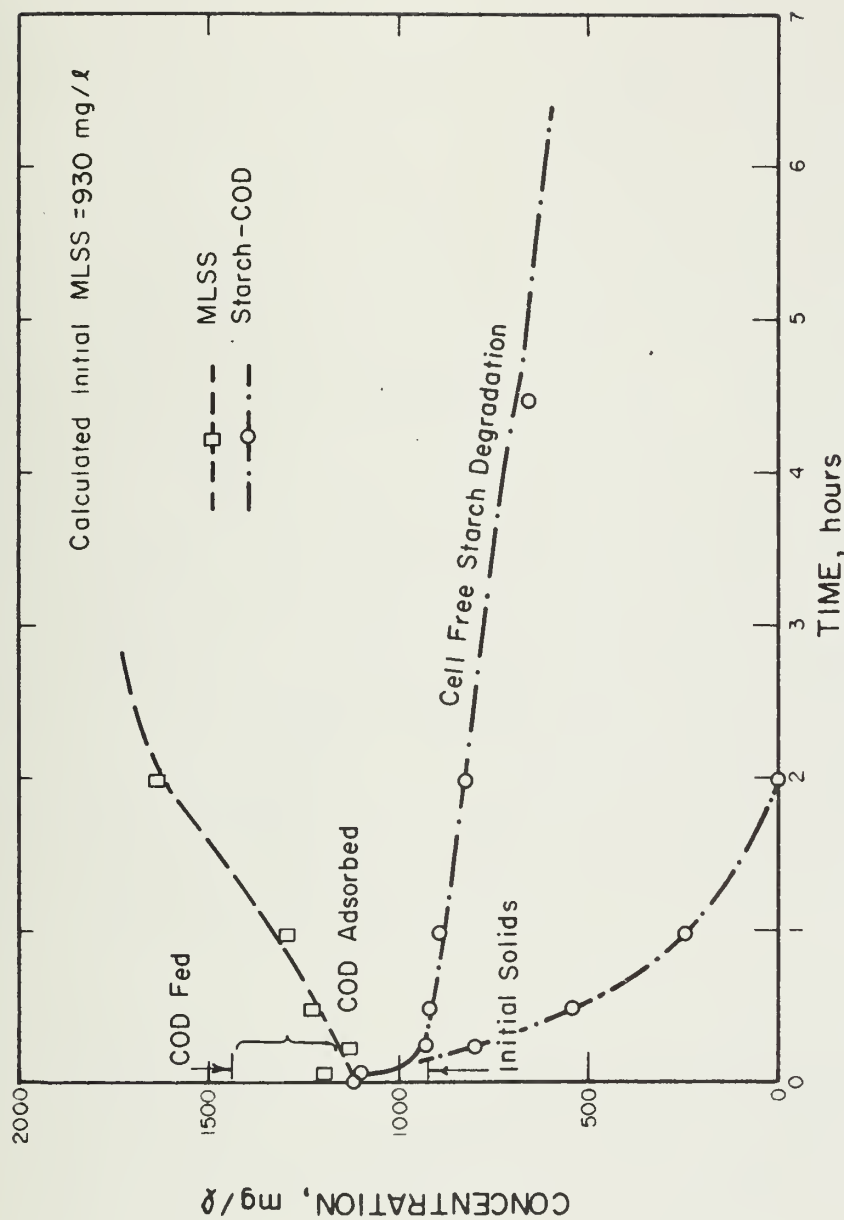


FIGURE 35 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN AN ACCLIMATED SYSTEM, WITH ACETATE BUFFER, UNDER AEROBIC CONDITIONS. F/M 1.40, TEMPERATURE 20°C

observed that the rate increased with an increase in temperatures and was not inhibited by substrate concentration. The use of acetate buffer at 20°C increased the rate about three-fold indicating possibly some calcium removal effect of the phosphate buffer.

It has been reported by Nomura et al. (74) that α -amylase of B. subtilis was liberated in the stationary and endogenous growth phase and very little extracellular α -amylase was obtained in the log growth phase.

This hypothesis was tested in the systems. All the cell-free starch-COD degradation tests reported earlier were obtained with sludges at or near the log growth phase. An experiment was performed to see if the filtrate from acclimated activated sludges in the endogenous phase would have a higher cell-free starch-COD degradation rate. Figure 36 shows the cell-free starch degradation with the filtrate from an acclimated activated sludge system in the endogenous phase. The cell-free starch degradation rate in the log growth phase obtained from Figure 12 is also plotted in Figure 36 for comparison. The rate of cell-free starch degradation is reported in Table 5 and was found to be much higher than the cell-free systems described earlier. However, the rate of starch-COD removal with the filtrate from acclimated potato starch sludge in the endogenous phase was still lower than the removal rate of whole cells.

6.3 Starch Substrate Removal in Non-Acclimated Aerobic Systems

These experiments were conducted to determine whether the enzyme system responsible for starch degradation in a non-acclimated activated sludge system was inducible, and also to study the nature of starch removal kinetics with these non-acclimated systems.

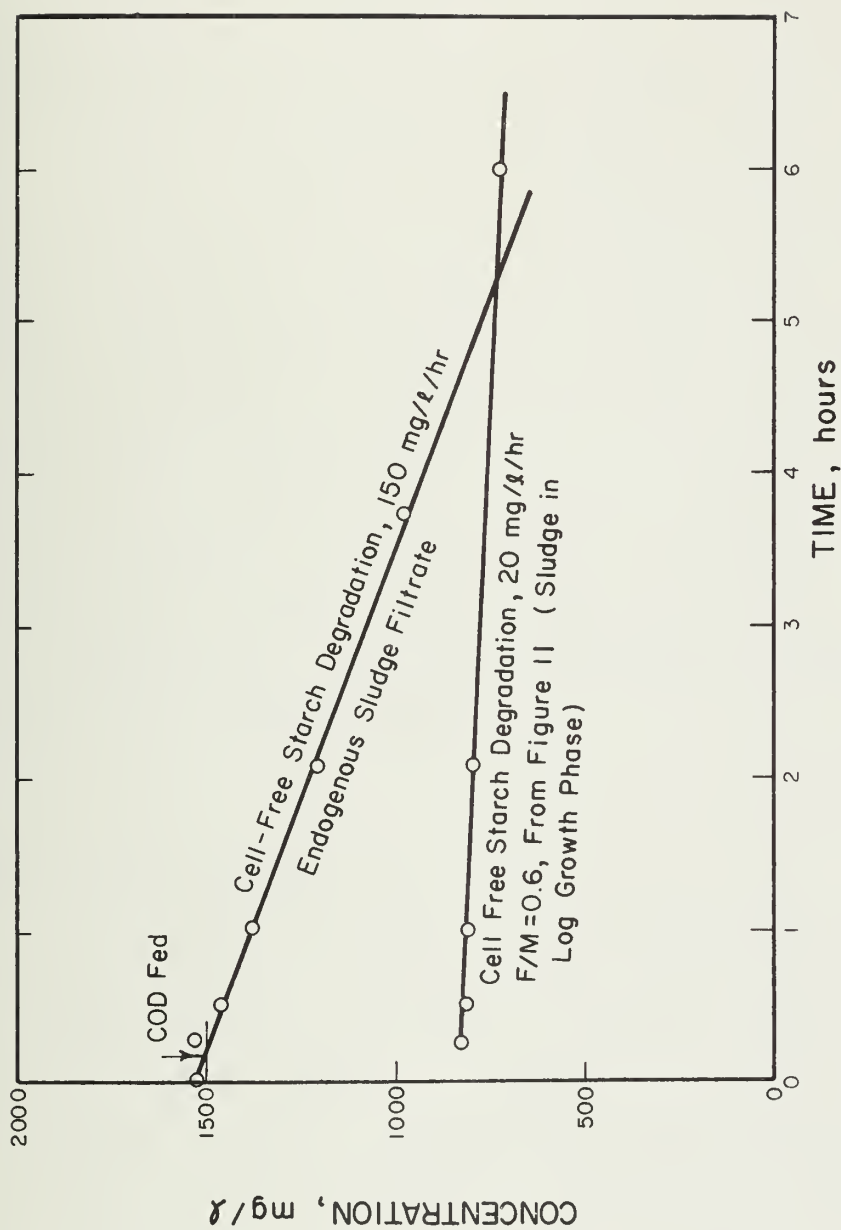


FIGURE 36 : STARCH -COD REMOVAL WITH ENDOGENOUS ACCLIMATED SLUDGE FILTRATES AT 20° C

6.3.1 Glucose Systems

In this experiment the sludge had been acclimated previously to glucose. Potato starch was added to this sludge and its removal characteristic at 20°C plotted as indicated in Figures 37 and 38. There was a marked lag in the total-COD and starch-COD removal rates at F/M ratio 2.54, after which the total-COD removal followed a linear rate and the starch-COD followed a logarithmic rate. At the lower F/M ratio (1.24), there was no lag and the rate of total-COD removal was linear, just as it had been in the acclimated sludge system. In this experiment, the starch-COD removal was logarithmic up to two hours. There was a change in the starch-COD removal rate after two hours in some cases, as shown in Figure 39.

Table 7 presents the starch removal rates, total-COD adsorbed, initial MLSS concentration and initial filtrate total-COD concentration with glucose acclimated sludge at 20°C. On comparing the data of starch removal by glucose acclimated sludge in Table 7 with that in Table 5 (for acclimated sludge) it becomes evident that starch-COD and total-COD rates were much lower with glucose acclimated sludge.

6.3.2 Maltose Systems

The sludge used in this system was acclimated to maltose. The starch removing characteristics of maltose-acclimated sludge are shown in Figures 39, 40 and 41. There was no lag observed in starch removal rate in the experiments. However, the total-COD removal characteristics in Figure 40 (F/M 1.20) were different from that obtained in the previous experiments mentioned earlier. After two hours during which the removal rate was linear, there was a break, and the removal rate was retarded from

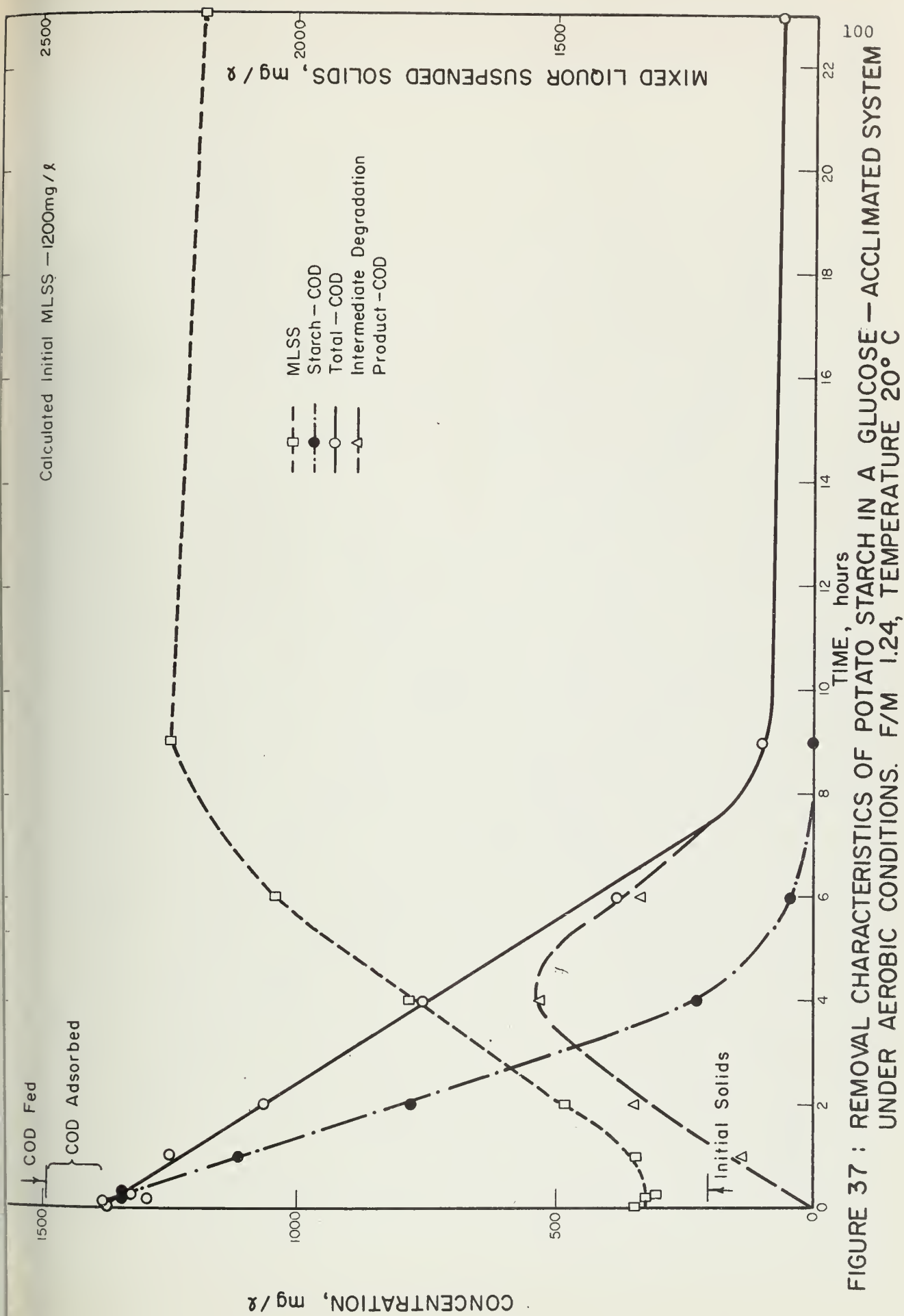


FIGURE 37 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN A GLUCOSE — ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS. F/M 1.24, TEMPERATURE 20° C

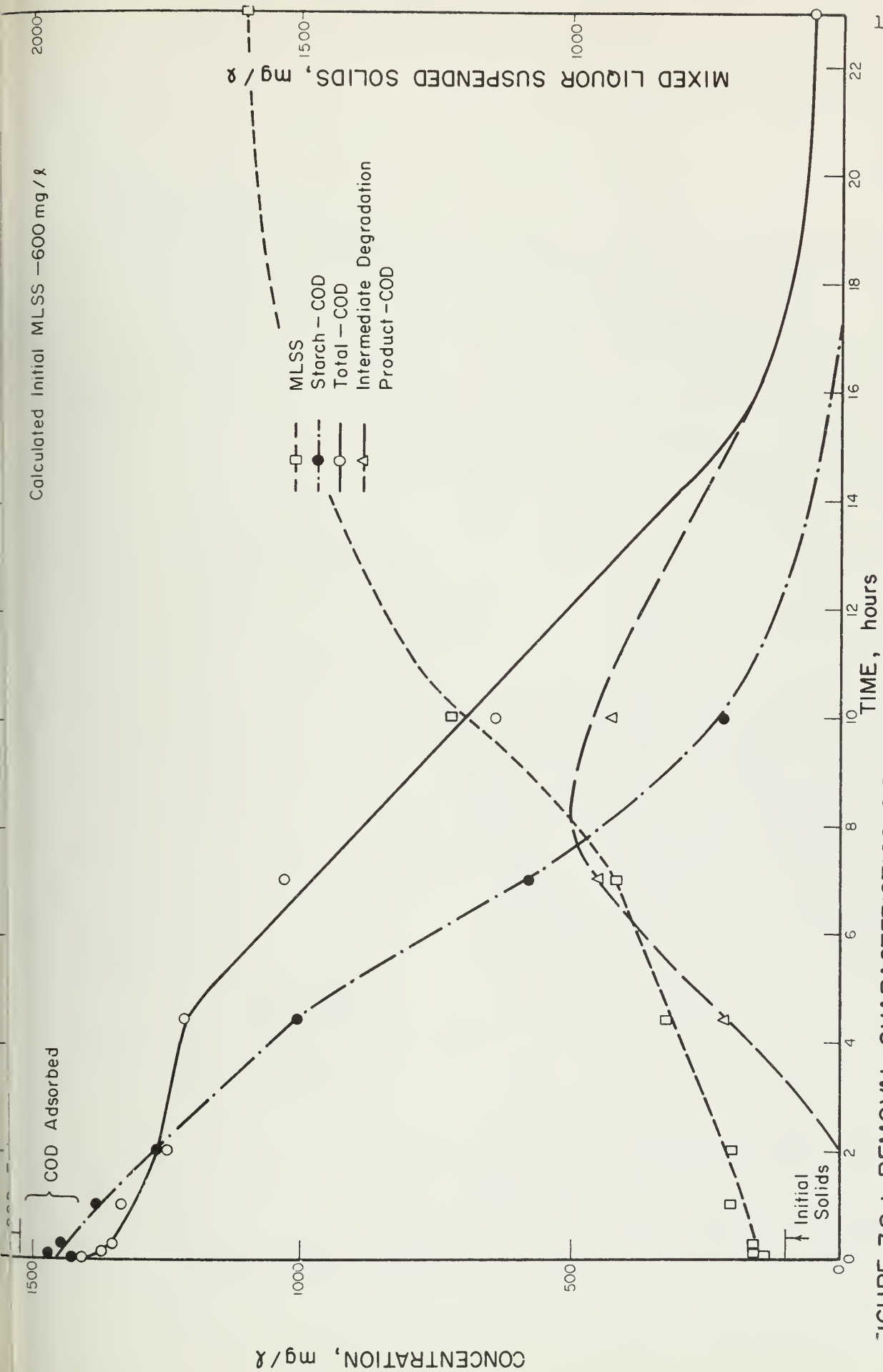


FIGURE 38 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN A GLUCOSE - ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS. F/M 2.54, TEMPERATURE 20°C

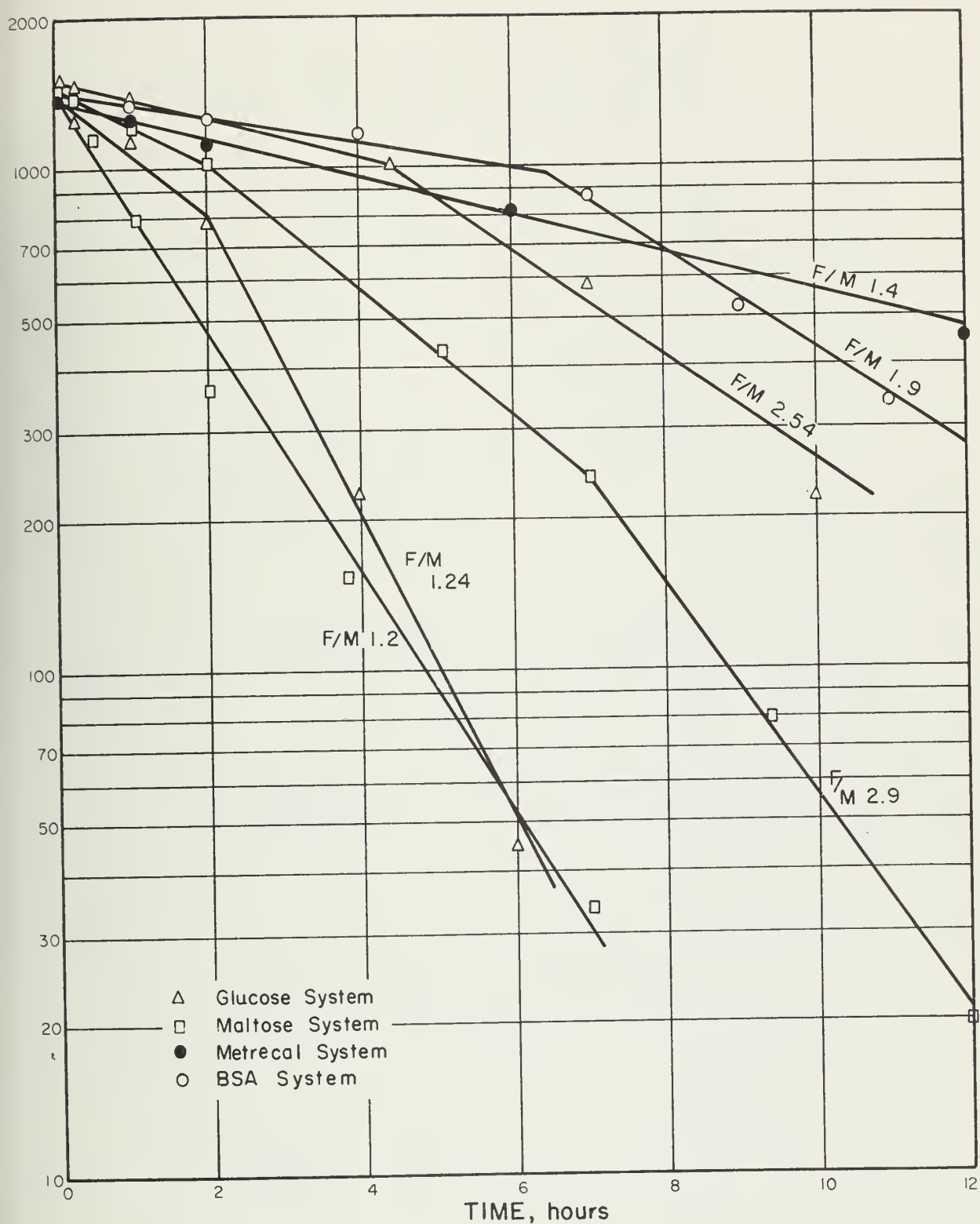


FIGURE 39: STARCH-COD REMOVAL IN NON-ACCLIMATED AEROBIC SYSTEMS AT 20° C

TABLE 7

STARCH REMOVAL BY NON-ACCLIMATED ACTIVATED SLUDGE SYSTEMS AT 20°C

F/M	Total-COD Removal Rate mg/l/hr	Starch-COD Removal Rate (hr) ⁻¹	Initial Total-COD Adsorbed mg/l	Initial MLSS mg/l	Initial Filtrate Total-COD Concentration mg/l	Remarks
mg/mg						
1.24	160	0.120	100	1200	1390	Glucose Acclimated Sludge
2.54	95	0.039	100	600	1400	
1.20	340	0.24	70	1250	1410	Maltose Acclimated Sludge
2.9	106	0.07	20	500	1400	
1.4	180	0.020	160	1100	1380	Metrecal Accl. Sludge
1.9	70	0.022	70	780	1400	BSA Acclimated Sludge
1.24	160	0.120	100	1200	1390	Glucose Acclimated Sludge = 1st Day = 2nd Day = 4th Day = 6th Day
1.17	275	0.161	370	1295	1140	
1.07	348	0.87	402	1420	1120	
1.14	365	1.154	120	1280	1340	

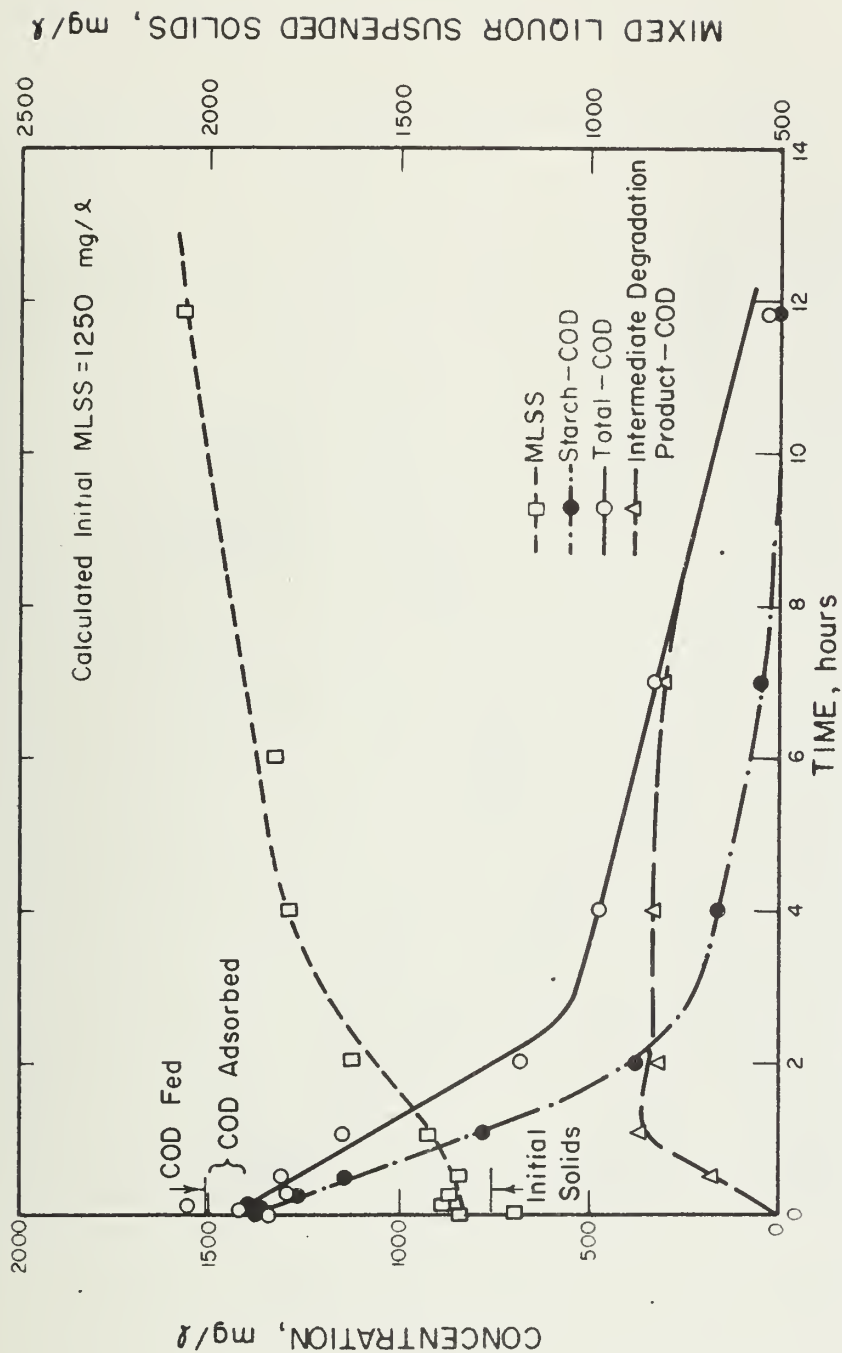


FIGURE 40 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN A MALTOSE-ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS. F/M 1.20, TEMPERATURE 20°C

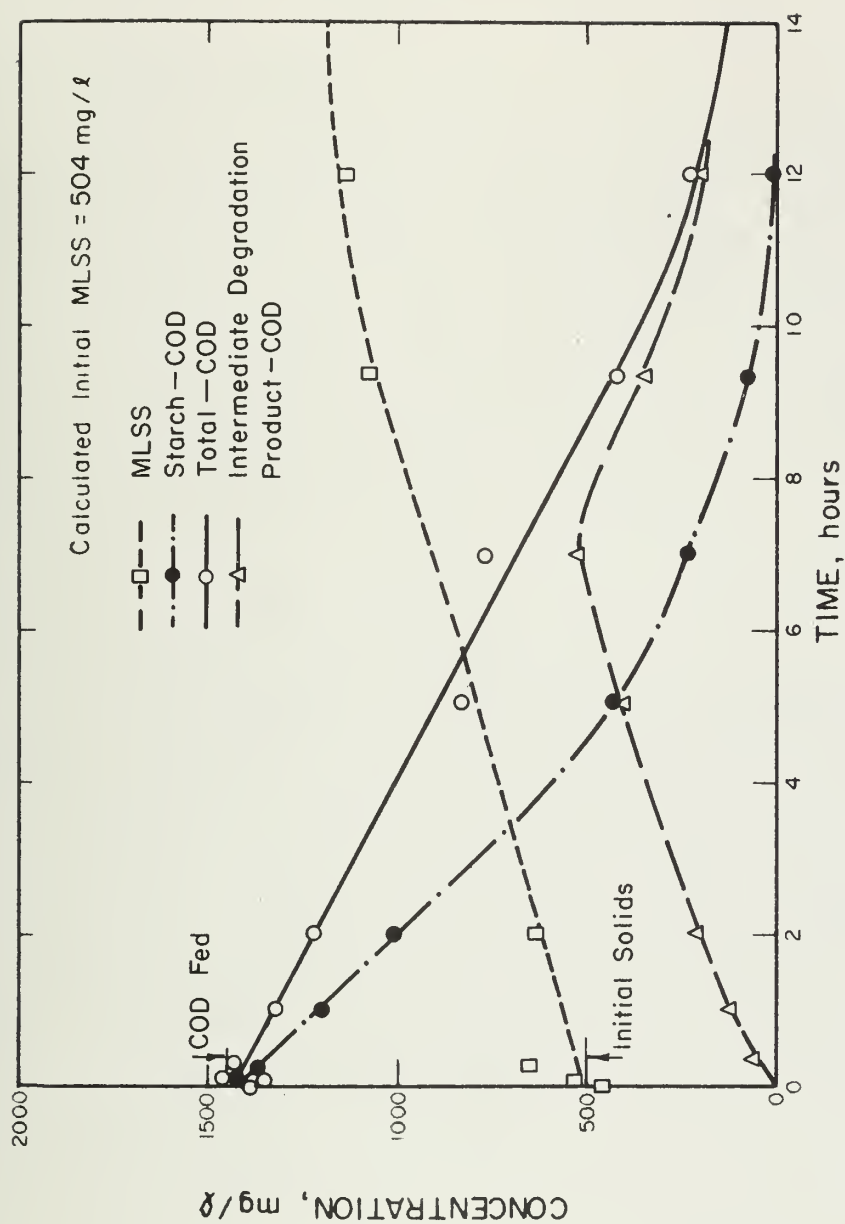


FIGURE 41 : REMOVAL CHARACTERISTICS OF POTATO STARCH IN A
MALTOSE-ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS.
F/M 2.9, TEMPERATURE 20°C

then on. The total-COD and starch-COD removal rates (Table 7) were lower than the rates of acclimated systems (Table 5), but were higher than those obtained for glucose acclimated sludge.

6.3.3 Metrecal System

Figure 42 represents the starch removal characteristics of the Metrecal acclimated sludge. There was a significant lag in the starch removal characteristics of the system, although after 23 hours of aeration, 97 percent of the fed substrate had been removed. The removal rate of starch-COD (as plotted on semi-log paper, Figure 39) was much lower than most of the systems at the same F/M ratio (Table 7).

6.3.4 Bovine Serum Albumin System

In this experiment the sludge had been acclimated to bovine serum albumin. The starch removal characteristics of this sludge are presented in Figure 43. There was a significant lag in both the starch-COD and total-COD removal rates, as in the case of Metrecal sludge. However, after a seven-hour lag total-COD removal occurred linearly. The starch-COD removal rate, Figure 39, was initially lower than the Metrecal sludge, but after the lag period, the rate improved and finally it was higher than that of the Metrecal system.

6.3.5 Acclimation of Glucose Sludge to Starch

It will be observed from Table 7 that, in general, non-acclimated sludges adsorbed less starch than acclimated sludges. In this experiment,

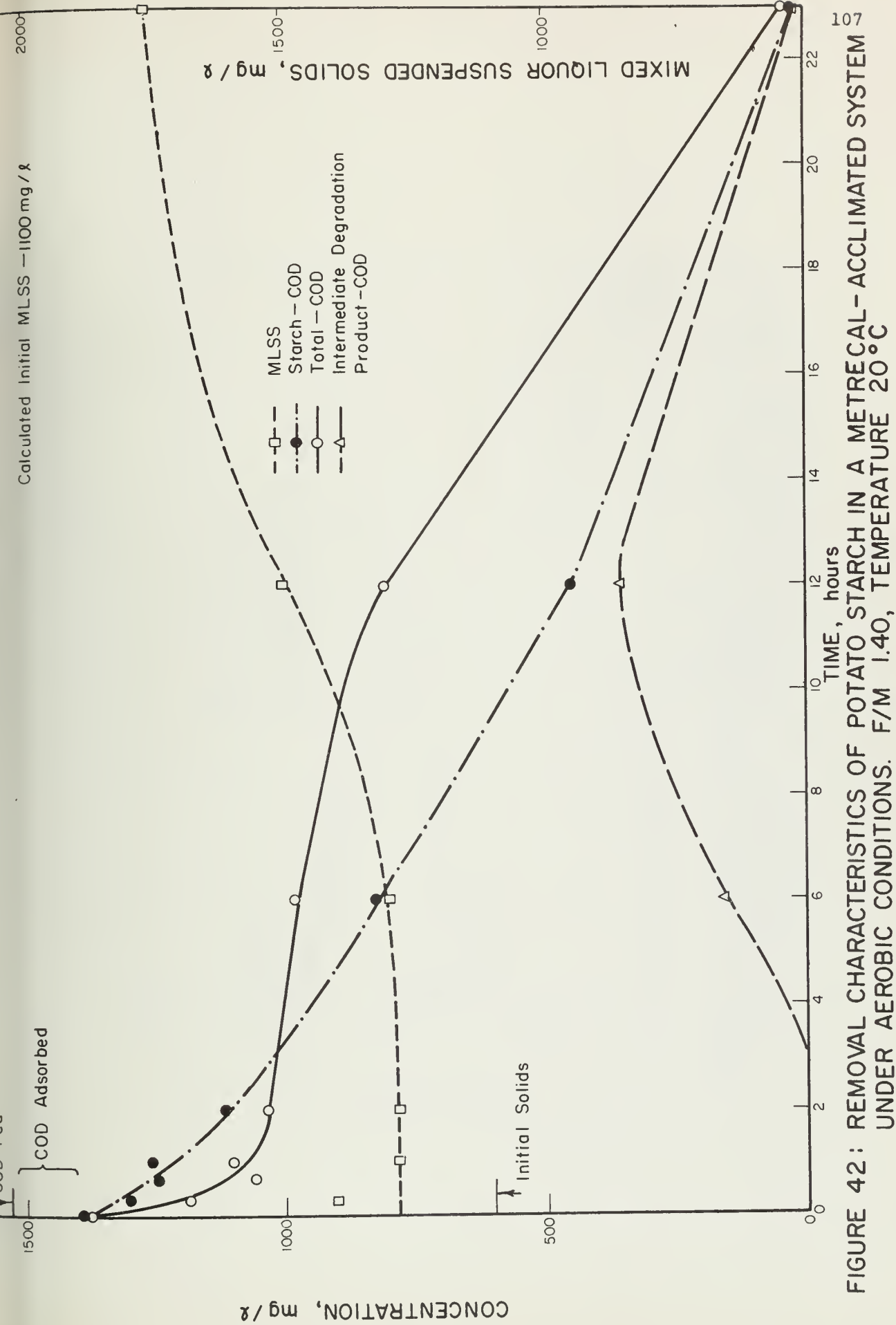


FIGURE 42: REMOVAL CHARACTERISTICS OF POTATO STARCH IN A METRECAL-ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS. F/M 1.40, TEMPERATURE 20°C

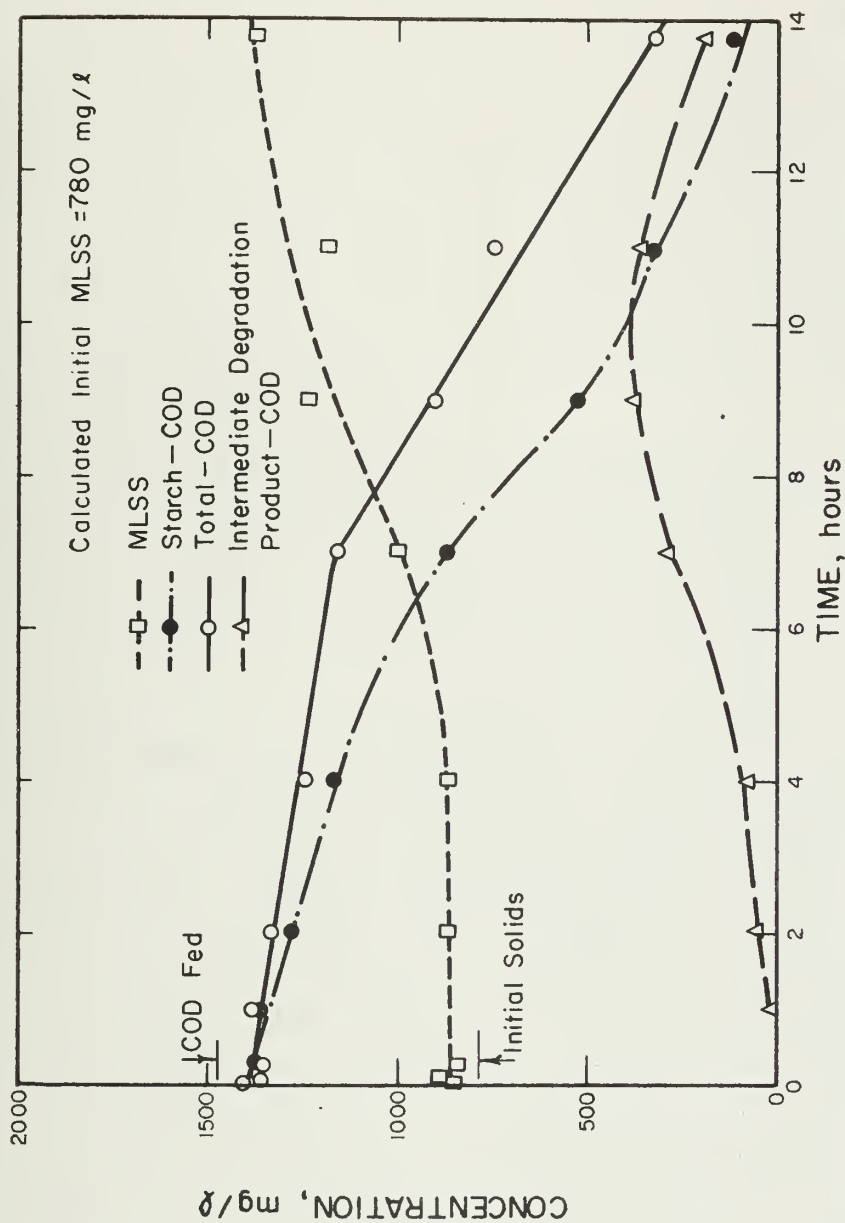


FIGURE 43: REMOVAL CHARACTERISTICS OF POTATO STARCH IN A BOVINE SERUM ALBUMIN-ACCLIMATED SYSTEM UNDER AEROBIC CONDITIONS. F/M 1.9, TEMPERATURE 20°C

glucose acclimated sludge was fed starch at 20°C on succeeding days until acclimation of the sludge to starch had occurred. The unit was operated with one-third mixed liquor wasted every day and other operational details were similar to those of the starch acclimation units.

Figures 44 through 46 indicate the changes in total-COD and starch-COD removal characteristics of the glucose sludge as it was being acclimated to starch. In six days, the starch-COD removal rate constant of the sludge increased from 0.12 to 1.154 (hour)⁻¹ at an average F/M ratio of 1.15 (Table 7). In fact, the sixth day starch-COD removal rate constant was slightly higher than the rate constant for acclimated sludge at 20°C at approximately the same F/M ratio.

The initial starch adsorption by the glucose sludge increased with acclimation and again decreased, as shown in Table 7. This was quite contrary to the expectation, as will be discussed later.

6.4 Acclimated Sludge Cell Wall Experiment

In earlier results it was pointed out that acclimated cell-free sludge filtrates had a low starch degrading capacity, although whole cells degraded starch at a rapid rate. This was observed even with acetate buffer, indicating that the removal of the calcium ion by phosphate buffer was not the reason for the low enzyme activity in cell-free sludge filtrates. The probability of inhibition of enzyme activity in sludge filtrates by high starch substrate concentration was also not found to be the case. At low starch substrate concentrations the enzyme activity in the sludge filtrate was also low. The enzyme activity in the endogenous sludge filtrate was much higher than in the sludge filtrates at the log growth phase.

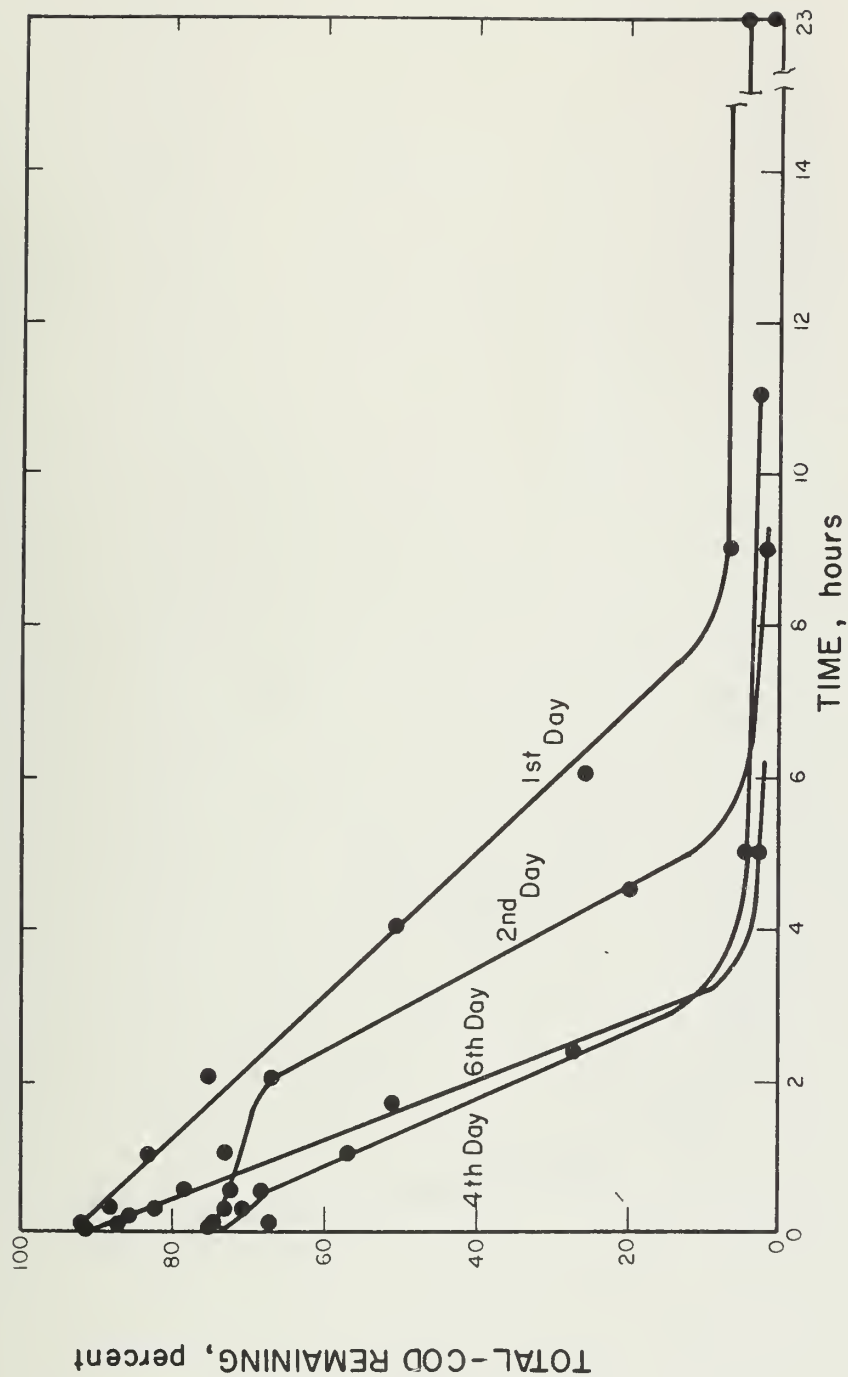


FIGURE 44 : CHANGE IN TOTAL—COD REMOVAL WITH ACCLIMATION OF
GLUCOSE-SLUDGE TO POTATO STARCH AT 20° C

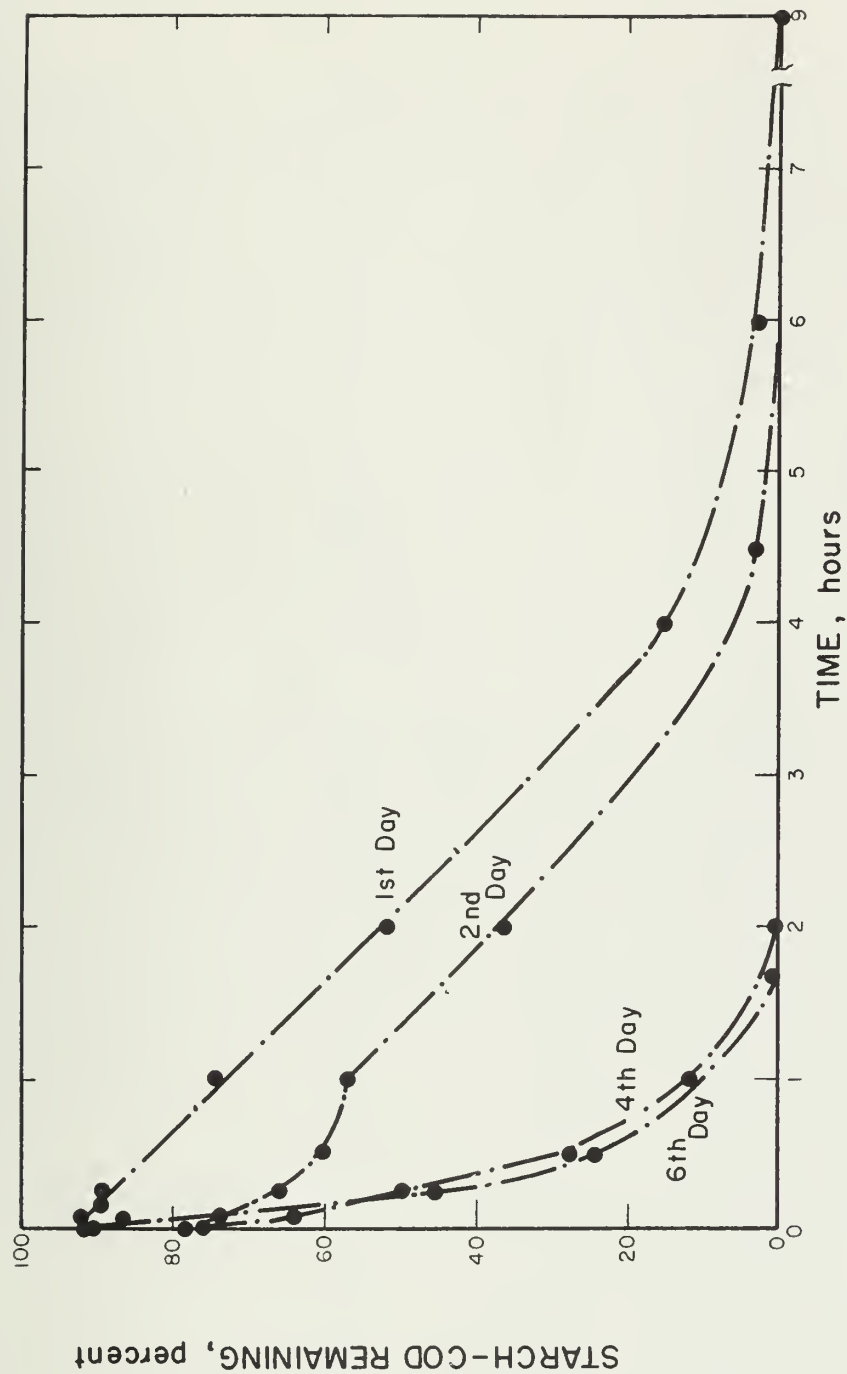


FIGURE 45 : CHANGE IN STARCH-COD REMOVAL WITH ACCLIMATION OF
GLUCOSE-SLUDGE TO POTATO STARCH AT 20° C

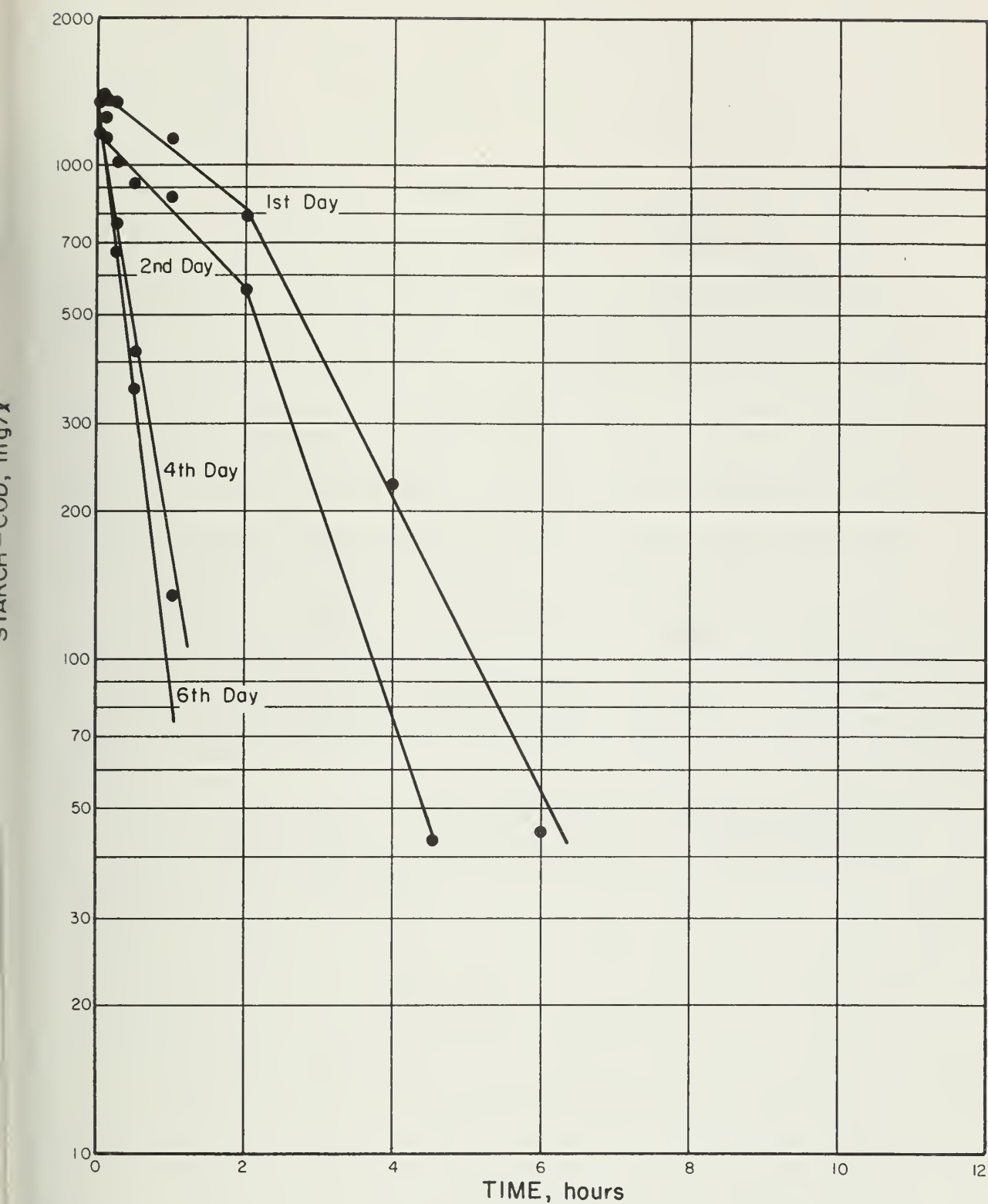


FIGURE 46: CHANGE IN STARCH-COD REMOVAL WITH ACCLIMATION OF GLUCOSE-SLUDGE TO POTATO STARCH AT 20° C

All these observations could be explained if it were assumed that the enzyme system responsible for starch degradation was confined to the outer cell surface, i.e., cell wall. During the endogenous phase, it is likely that there was an excretion of these enzymes into the medium, and therefore, the activity of the starch degrading enzyme was much higher.

The isolated cell wall and soluble components of the cell were tested separately for starch-COD removal characteristics, as depicted in Figures 47 and 48. The starch-COD removal rate of a whole cell sludge, a part of which was used for cell wall preparation, has also been presented. Table 8 gives the removal rates and amount of starch adsorbed by the cell and its components. The starch-COD removal rates were expressed per gram per liter of protein in the system in order to compare the various cell fractions on an equal basis. The rate of removal of starch-COD by the cell wall and soluble component fractions was much higher than that obtained for whole cells. The higher starch-COD removal rate of the cell wall suspension as compared to whole cell does indicate a cell surface bound enzyme system.

The adsorption of starch on the cell wall suspension was also observed as with the whole cells.

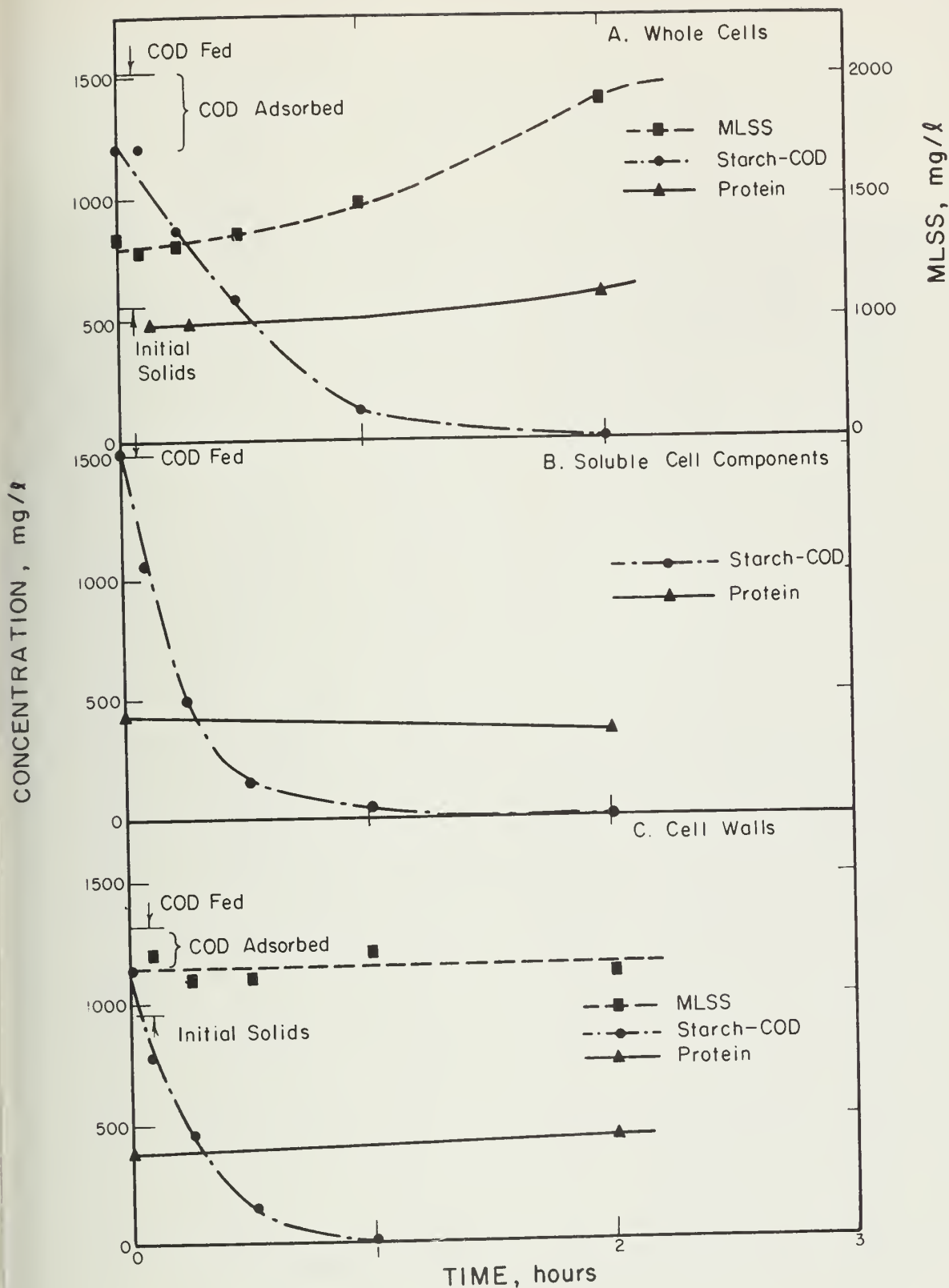


FIGURE 47: REMOVAL CHARACTERISTICS OF STARCH IN AN ACCLIMATED AEROBIC SYSTEM BY CELLS AND THEIR COMPONENTS AT 20° C

STARCH-COD, mg/l

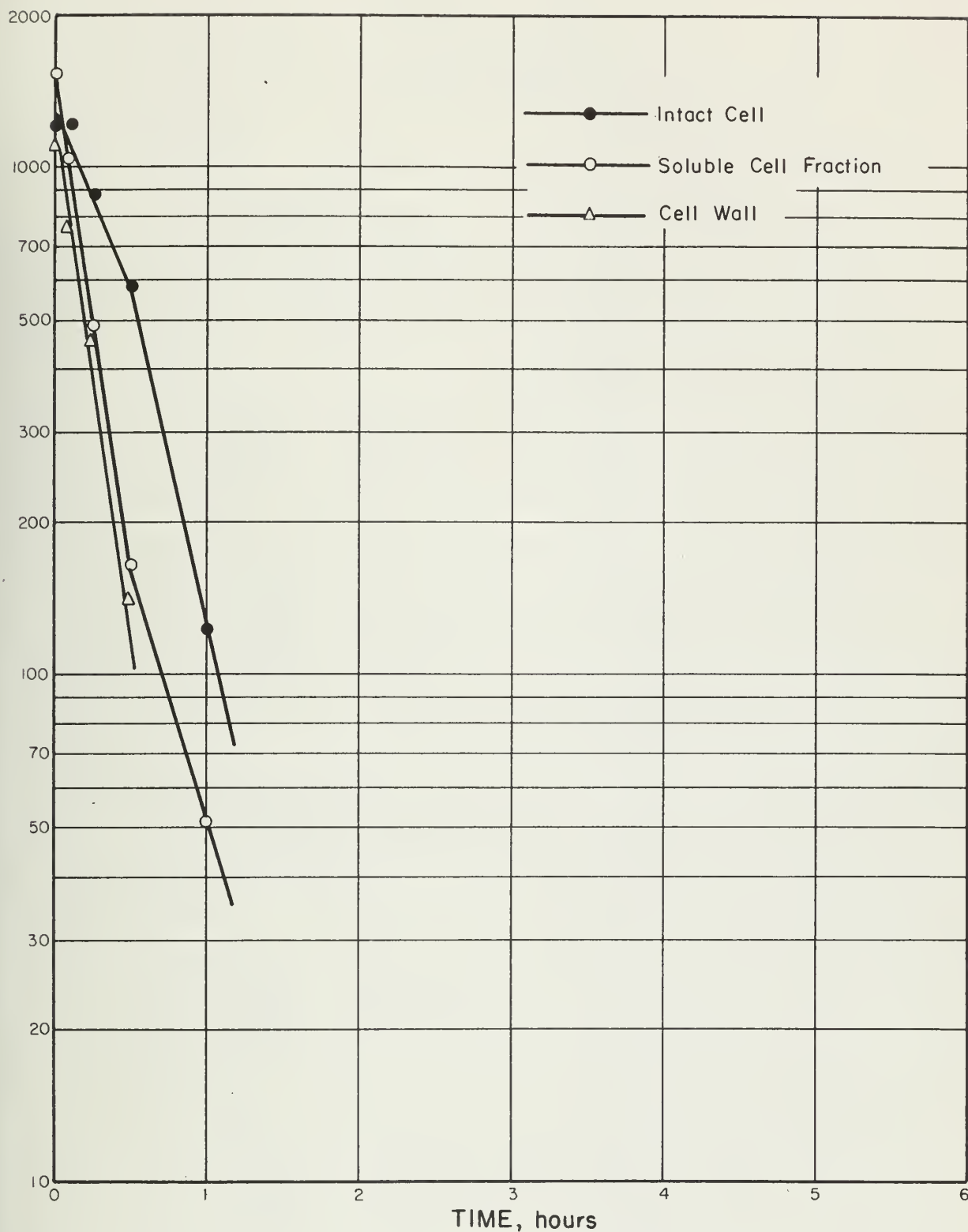


FIGURE 48 : REMOVAL CHARACTERISTICS OF STARCH-COD IN AN ACCLIMATED AEROBIC SYSTEM BY CELLS AND THEIR COMPONENTS AT 20° C

TABLE 8

STARCH REMOVAL BY ACCLIMATED SLUDGE CELLS AND ITS COMPONENTS AT 20°C

F/M	Starch-COD Removal Rate	Average Protein	Starch-COD Removal Rate per g/l Protein	Initial Starch-COD** Adsorbed	Initial MLSS	Initial Filtrate Starch-COD** Concentration	Remarks
mg/mg	(hr) ⁻¹	mg/l	(hr) ⁻¹ (g/l) ⁻¹	mg/l	mg/l	mg/l	
1.42	0.65	470*	1.39	300	1060	1200	Whole Cell
1.39	1.894	410	4.62	200	950	1120	Cell Wall Suspension
-	1.896	390	4.85	-	-	1510	Soluble Cell Component

* For whole cells the protein content was at the start of the experiment since it changed with time due to cell growth.

** Total-COD was not determined, only starch-COD was determined.

7. DISCUSSION OF RESULTS

It will be evident from the experimental results and the scope of the investigation that the characteristics of starch removal in an activated sludge system are dependent on many variables and the most important of these, it is believed, have been dealt with here in detail. However, there are some variables, like pH, presence of other substrates, nutrient deficiency, etc., which may also affect the removal of starch in an activated sludge system and have not been studied.

7.1 Starch Removal in Acclimated Systems

7.1.1 Kinetics of Starch Removal

The total-COD removal kinetics in all the systems studied with acclimated sludge followed a linear rate until a low COD level was reached similar to the results obtained by Wuhrmann (37). Eckenfelder and O'Connor (34) have also stated that at high substrate concentrations, linear kinetics were obtained for a few industrial wastes. The total-COD removal represents the amount of starch degradation products assimilated by the sludge at any time, although total-COD itself represents the amount of undegraded starch and starch degradation product-COD. In the present study there was an initial lag in some cases, especially with high F/M ratios, which was followed by the linear removal kinetics. This lag was due to a quantitative shock of high substrate concentration per cell. These cells had been acclimated to potato starch at an F/M ratio of about 1.13 and any change of this ratio produced this lag. There was no lag in Figures 12, 13, 14 and 22, where the F/M ratio was very close to that of the acclimation units (Appendix B).

The rate of total-COD removal is plotted for various values of F/M and initial solids concentration at different temperatures in Figures 49 and 50 respectively. The total-COD removal rate was dependent on the initial solids concentration and not on F/M ratio. This was quite evident when the removal rates for constant MLSS system at 20°C were plotted. In Figure 49, the rate of removal of total-COD remained practically constant in the case of constant MLSS and variable substrate system with a change in F/M. If total-COD removal rate was dependent on the F/M then there should have been an increase in the removal rate with an increase of $(F/M)^{-1}$. Since this rate was dependent on initial mixed liquor suspended solids, in Figure 50, there was little variation of the rate at a constant sludge concentration system. The removal of total-COD represents direct assimilation of the starch degradation products by the cell. This total-COD removal rate was found to be independent of substrate concentration, because of the linear substrate removal kinetics observed in most of the experiments. Therefore, the proportional variation of the total-COD removal rate due to change in $(F/M)^{-1}$ would be expected to be dependent upon the initial mixed liquor suspended solids. This would perhaps mean that the permease systems responsible for uptake of starch degradation products were constant for a particular sludge concentration, and were not dependent on the amount of substrate present. At 1200 mg/l sludge concentration the rate of removal of total-COD was about 400 mg/l/hr at 20°C regardless of the concentration of starch (Figure 50). If the substrate concentration was 200 mg/l, it would take only half an hour for the 1200 mg/l sludge concentration to remove it from the system, and if the substrate concentration was 1200 mg/l, it would take about 3 hours to remove it from the system.

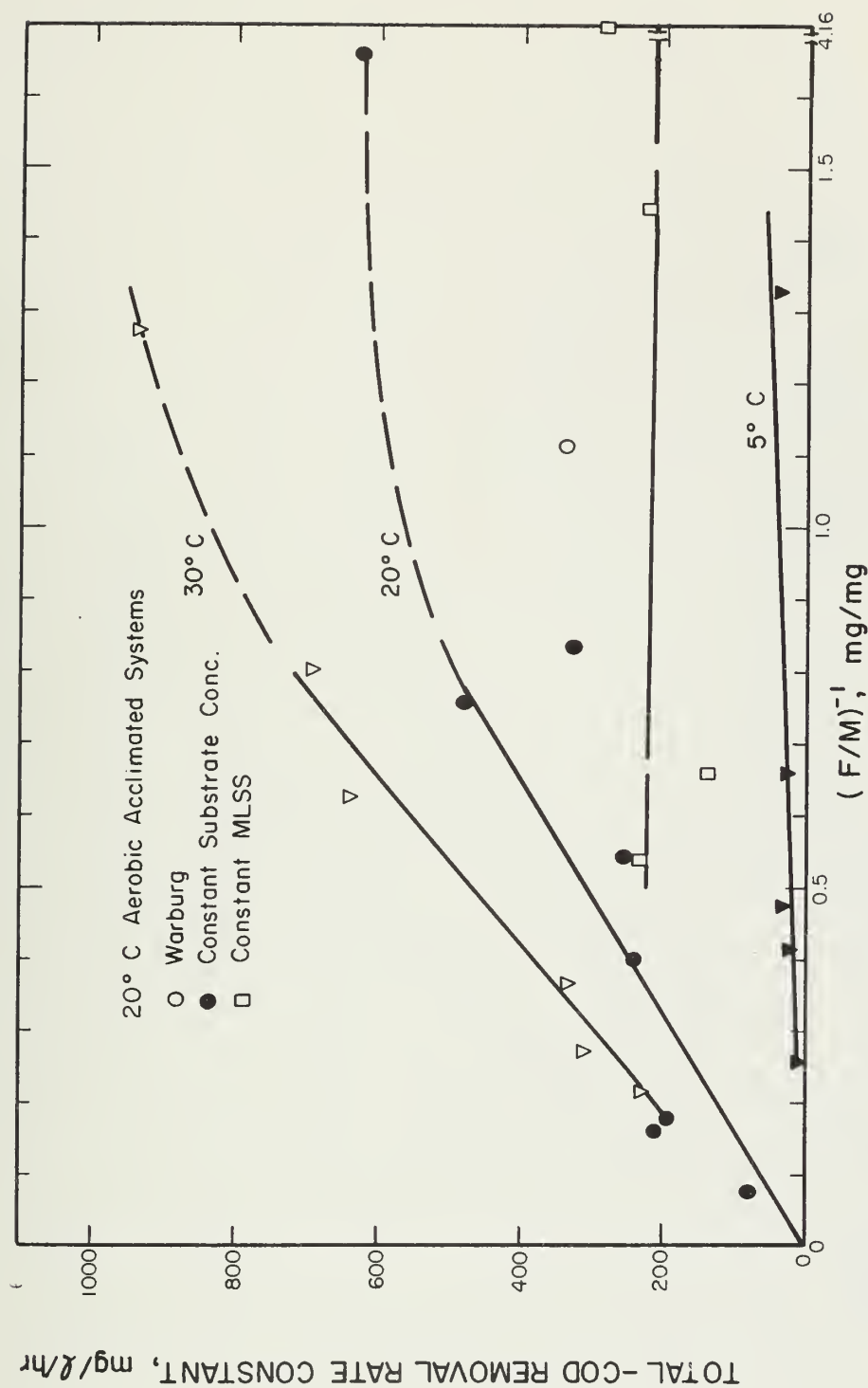


FIGURE 49: RATE CONSTANT OF TOTAL—COD REMOVAL AT DIFFERENT F/M AND TEMPERATURES FOR AN ACCLIMATED SYSTEM

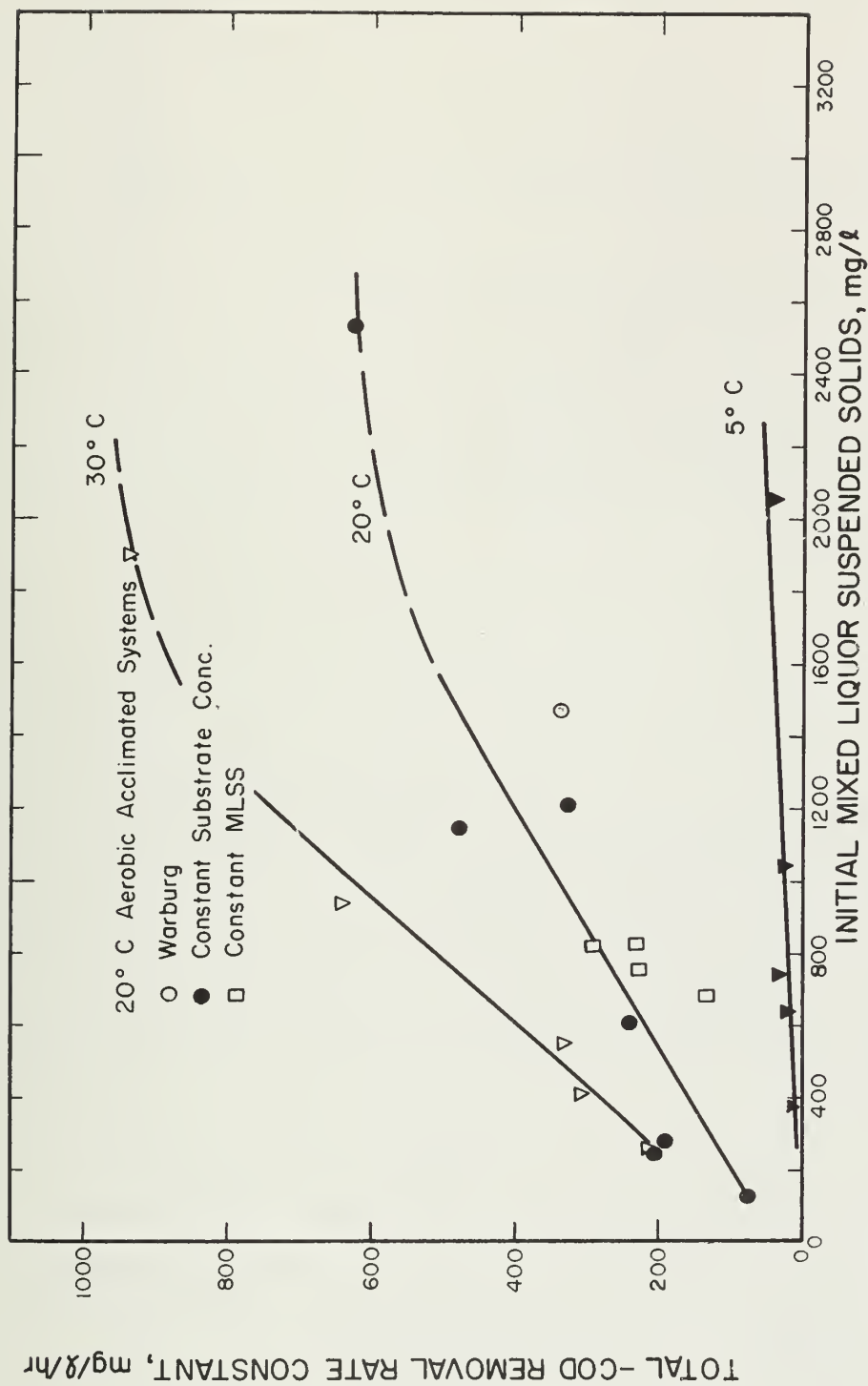


FIGURE 50: RATE CONSTANT OF TOTAL—COD REMOVAL AT DIFFERENT INITIAL MLSS AND TEMPERATURES FOR AN ACCLIMATED SYSTEM

The equation of total-COD removal, neglecting the initial lag, can be written as

$$\frac{dL}{dt} = -k_t \quad k_t = \text{Total-COD removal rate constant}$$

or
$$(L_o - L_t) = k_t(t - t_o) \quad 14$$

The k_t itself was found to be dependent on temperature (which is discussed later) and initial solids concentration. At constant temperature,

$$k_t = b(S_o) \quad 15$$

where b = constant

S_o = initial solids concentration, mg/l

The value of "b" for different temperatures can be obtained from the slope of the linear portion of the curve in Figure 50. Table 9 presents the value of "b" at different temperatures. Equation 14 is very similar to that deduced earlier, Equation 4a, for constant temperature conditions.

TABLE 9
VALUES OF "b" AND "c" AT DIFFERENT TEMPERATURES

Temperature °C	"b" mg COD/hr/g sludge	"c" mg/mg/hr
5	28	0.154
20	315	1.04
30	575	1.11

The values of linear removal rates "b," expressed in mg BOD/hr/g sludge for pharmaceutical, brewery, refinery and spent sulfite liquor wastes were reported to be 200, 100, 131 and 107 respectively (34). If the COD rates obtained in Table 9 are converted to BOD by multiplying by 0.65, the 20°C value would be 204 mg BOD/hr/g sludge.

It will be also evident from Figure 50, at 20°C and 30°C, that after a mixed liquor suspended solids concentration of 1200 mg/l, the linearity of the curve was lost and the curve flattened out. This cannot be explained on the basis of enzyme unsaturation with an increase in sludge mass, the substrate remaining constant. If that were the case we would have obtained much lower total-COD removal rate in the case of constant MLSS system F/M 0.24, Figure 28. Thus, even at higher mixed liquor suspended solids concentrations we would expect high total-COD removal rates. It will be evident later that starch-COD removal rates at high values of $(F/M)^{-1}$ are less than those predicted by the linear relationship shown in Figure 51. This lower degradation rate of starch at higher initial solids concentration (since F is constant) may also affect adversely the total-COD removal rate since the latter is dependent on the starch degradation. The other possibility for lower removal rates at high solids concentrations could be that the high oxygen demand of the cells was not satisfied completely by the aeration system.

Wuhrmann (37) pointed out that the rate of substrate removed was proportional to the sludge concentration. This relationship was found to be true even up to 7000 mg/l sludge concentrations for lactose. However, he pointed out that this would be true only within the range of sludge concentration in which an adequate oxygen and food supply to each individual cell was maintained.

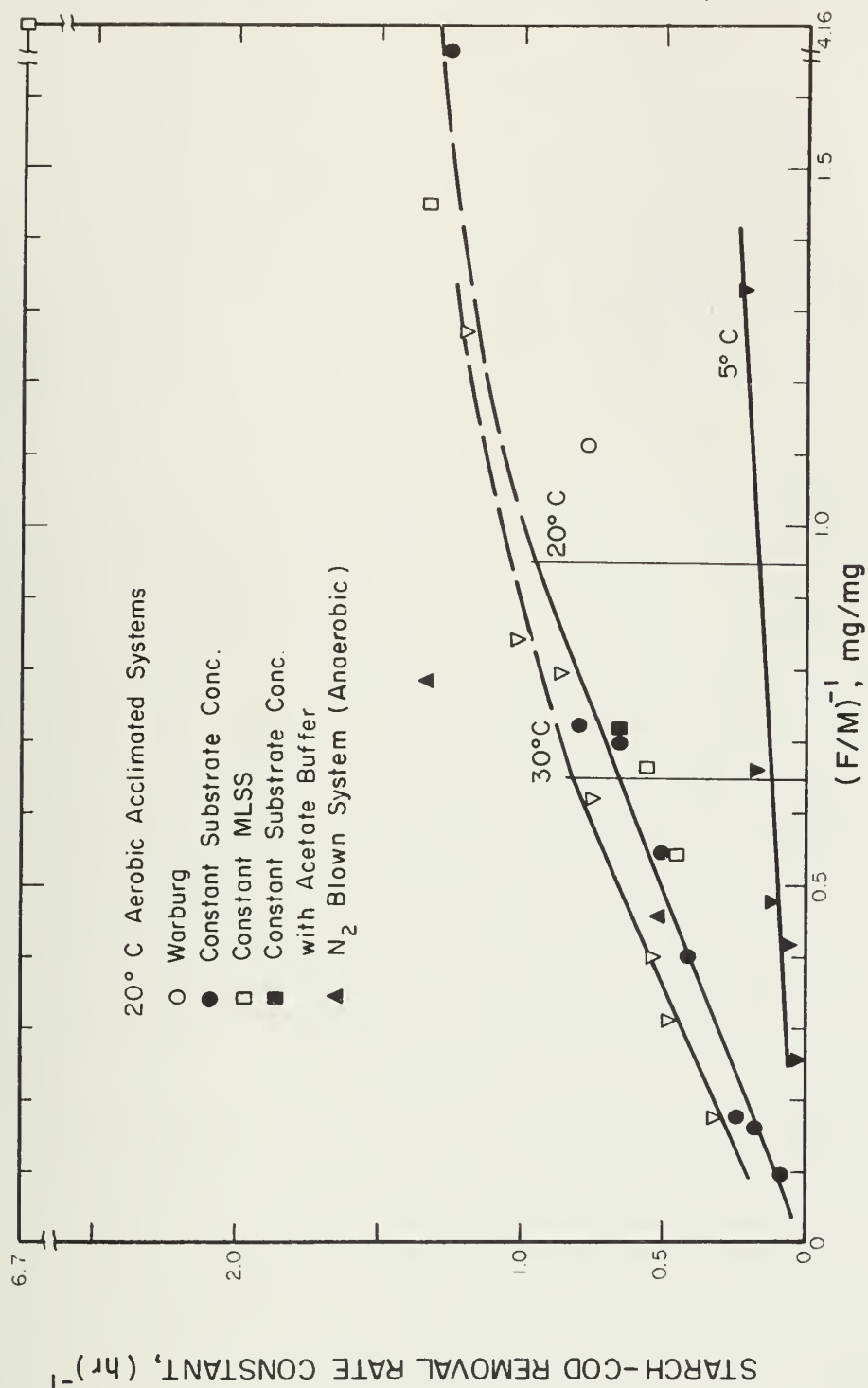


FIGURE 51 : RATE CONSTANT OF STARCH-COD REMOVAL AT DIFFERENT F / M AND TEMPERATURES FOR AN ACCLIMATED SYSTEM

In Figures 50 and 51, data from an experiment using the Warburg apparatus is also plotted. The removal rate in the Warburg respirometer (Figure 20) was lower than that of the activated sludge system. This was probably due to different mixing and aeration of the two systems. It will be observed from Figures 13 and 20 that the total carbohydrate removal rate was very similar to total-COD removal rate, and as has been mentioned earlier, in later experiments the total carbohydrate test was abandoned since all information could be obtained just as well with the COD test. However, it would be of interest to note that there was no accumulation of intermediate degradation product other than carbohydrates during the utilization of starch. Apparently, the starch is degraded to smaller carbohydrate molecules which are removed from the system by cellular assimilation. In Figure 20, the oxygen uptake rate was high and fairly constant for the period the total-COD removal was linear. After most of the substrate had been removed from the medium, the oxygen uptake proceeded at a slower rate, which was still much higher than the endogenous rate. This high oxygen utilization rate after the removal of substrate from the medium would indicate the utilization of cellular storage products by the cell for synthesis and energy.

Further, it will be noticed from Figures 49 and 50 that there was quite a large scatter of points, especially at 20°C. This may be attributed to the difference in active cell mass in the systems. The rate of removal of substrate in any system would be dependent on the active cell mass and not on the total suspended solids. Thus, if the active cell mass as represented by sludge volatile solids or organic nitrogen content of the sludge (75) was used, there would have been less scatter of points in the Figures 49 and 50.

Starch removal in the activated sludge system would be by amylolytic enzymes. These enzymes would degrade the starch to smaller size saccharides which could be transported by the cell permease systems for assimilation. The kinetics of the amylase action on starch is very complex (76) because of the following reasons:

(i) starch is not a homogeneous chemical compound. It is a mixture of linear amylose and branched amylopectin, which may vary in degree of branching and polymerization.

(ii) the amylolytic reaction is in itself complicated owing to the polymeric nature of substrate. The splitting of each glucosidic bond yields new products which may in turn act as substrate for the same enzyme molecule.

(iii) in the random attack peculiar to α -amylase, the products will vary considerably in molecular weights, at least initially, so the enzyme will be solicited by a family of substrates for which it will have quite different affinities.

Adding to the complexity will be the numerous microorganisms of the activated sludge systems having enzymes with different affinities for starch. The rate of permeation of starch degradation products into the cells may also influence the extent of amylolytic action.

Therefore, with these difficulties in mind, attempts were made to evolve a simple parameter of comparison of initial starch removal rates of various activated sludge systems.

The starch-COD removal kinetics in each case were plotted on rectangular and semi-log paper. It was observed in all cases that the initial rate was linear on semi-log plot indicating that the rate of starch-COD removal was proportional to the starch-COD concentration. However, at

later stages in some cases, there was divergence from this logarithmic relationship. This divergence was attributed to the complexity of reaction and perhaps change in substrate nature by the enzyme action.

The activated sludge substrate removal by the two phasic equations developed in an earlier chapter are not applicable for starch-COD removals because the starch molecules removed from the system are actually converted to smaller size carbohydrate molecules and are not directly utilized by the cell. These smaller size carbohydrate molecules still exert oxygen demand and are directly utilized by the cell.

The simple relationship developed for initial rate of starch-COD removal from the Figures 10, 19 and 27 was

$$\frac{dL}{dt} = -KL \quad 16$$

which on integration gave

$$\log \frac{L_o}{L_t} = k_s (t - t_o) \quad 17$$

$$k_s = \text{starch-COD removal rate constant; } k_s = \frac{K}{2.303}$$

The k_s values for acclimated activated sludge systems are reported in Tables 4, 5 and 6 for different temperatures. Figures 51 and 52 represent the relationship of starch-COD removal, at different temperatures, with $(F/M)^{-1}$ and initial sludge concentration respectively.

It will be seen that at constant substrate systems, the rate of starch-COD removal was dependent on initial mixed liquor suspended solids (and $(F/M)^{-1}$ because F was constant). However, the data of constant mixed

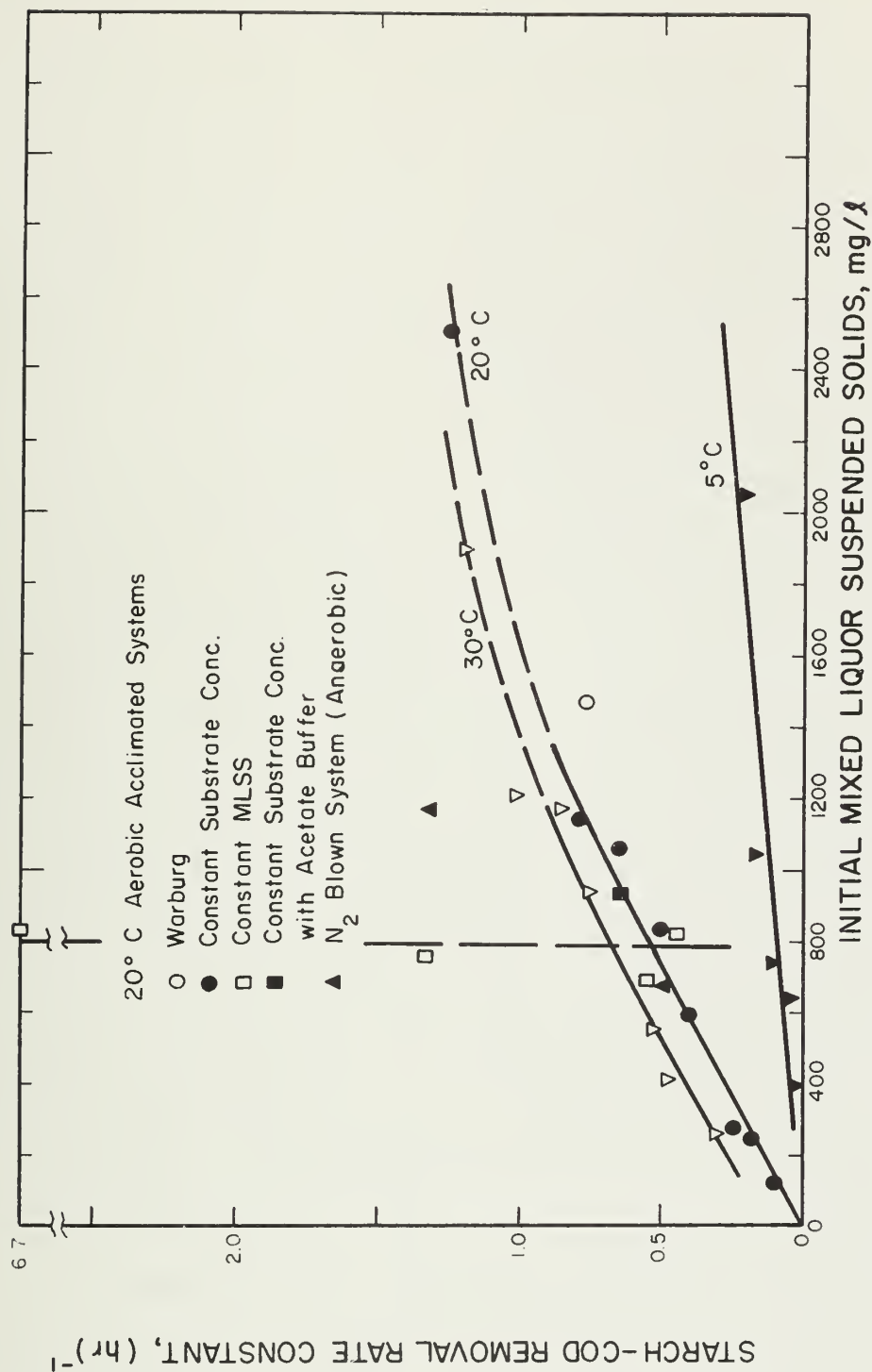


FIGURE 52 : RATE CONSTANT OF STARCH-COD REMOVAL AT DIFFERENT INITIAL MLSS AND TEMPERATURES FOR AN ACCLIMATED SYSTEM

liquor suspended solids systems (Figures 28 through 32) indicated that starch-COD removal was not dependent on initial solids but on the F/M, as the rate of starch-COD removal at constant sludge concentration in Figure 52 was quite independent of the sludge concentration. The dependence of starch-COD removal rate on the F/M ratio rather than the initial sludge concentration would indicate that the enzymatic action responsible for the starch degradation involved both the sludge and substrate concentrations. The initial removal rate of starch-COD has been found to be dependent on the substrate concentration because of the logarithmic removal characteristics in most of the experiments. Also, the starch-COD removal rate changed with the alteration in the initial solids concentration in the experiments with constant substrate level. Therefore, it would be expected that the starch-COD removal rate would be dependent on both the substrate and sludge concentrations or on the F/M ratio. At high $(F/M)^{-1}$ values there was a divergence from the linear plots for 20°C and 30°C in Figure 51, as also observed in Figures 49 and 50 with total-COD removal rates. The plausible explanation for this decrease in the starch-COD degrading rate at high $(F/M)^{-1}$ values was that the enzymes of the system were not saturated with substrate and so the overall rate was lower. This divergence from linear relationship in Figure 51 for 20°C was at $(F/M)^{-1}$ of 0.95 and of 0.65 for 30°C. The dependence of starch-COD removal rate on F/M rather than on initial solids was different from that observed in the case of total-COD removal rate. However, the basic difference between the two removal phenomena must be emphasized in order to understand the variation. Total-COD can only be removed by assimilative processes of the cell, whereas the starch-COD removal need not be due to assimilation, but simply to degradation into smaller fragments giving negative starch-iodine test. Although

both processes are enzymatic, the starch-COD removal rate is perhaps independent of total-COD removal rate. This conclusion is based on the anaerobic experiments discussed later.

The starch-COD removal rate constant k_s is, therefore, dependent on the inverse of F/M ratio at constant temperatures. So,

$$k_s = c(F/M)^{-1} \quad 18$$

The values of constant of proportionality "c" at different temperatures are presented in Table 9, and were obtained directly from the linear portion of curves in Figure 51. The relation between starch-COD removal rate of the acclimated activated sludge and the total-COD removal rate has been presented in Figure 53 for different temperatures. At a particular temperature there was a more or less linear relationship between the two provided the substrate level was maintained constant. If the mixed liquor suspended solids were kept constant instead, then the starch-COD removal rate was practically independent of total-COD removal rate. This has been explained earlier. The total-COD removal rate was dependent on initial solids whereas starch-COD removal was dependent on F/M. In the constant MLSS system the total-COD removal rate was constant but starch-COD removal rate varied with changed F/M ratios.

Attempts were also made to plot the starch-COD removal rates in the Michaelis-Menten relationship at 20°C, with a constant sludge concentration, Equation 19. The linear form of the Equation 9 was used, which is

$$\frac{1}{v} = \frac{L_K}{V} \cdot \frac{1}{L_t} + \frac{1}{V} \quad 19$$

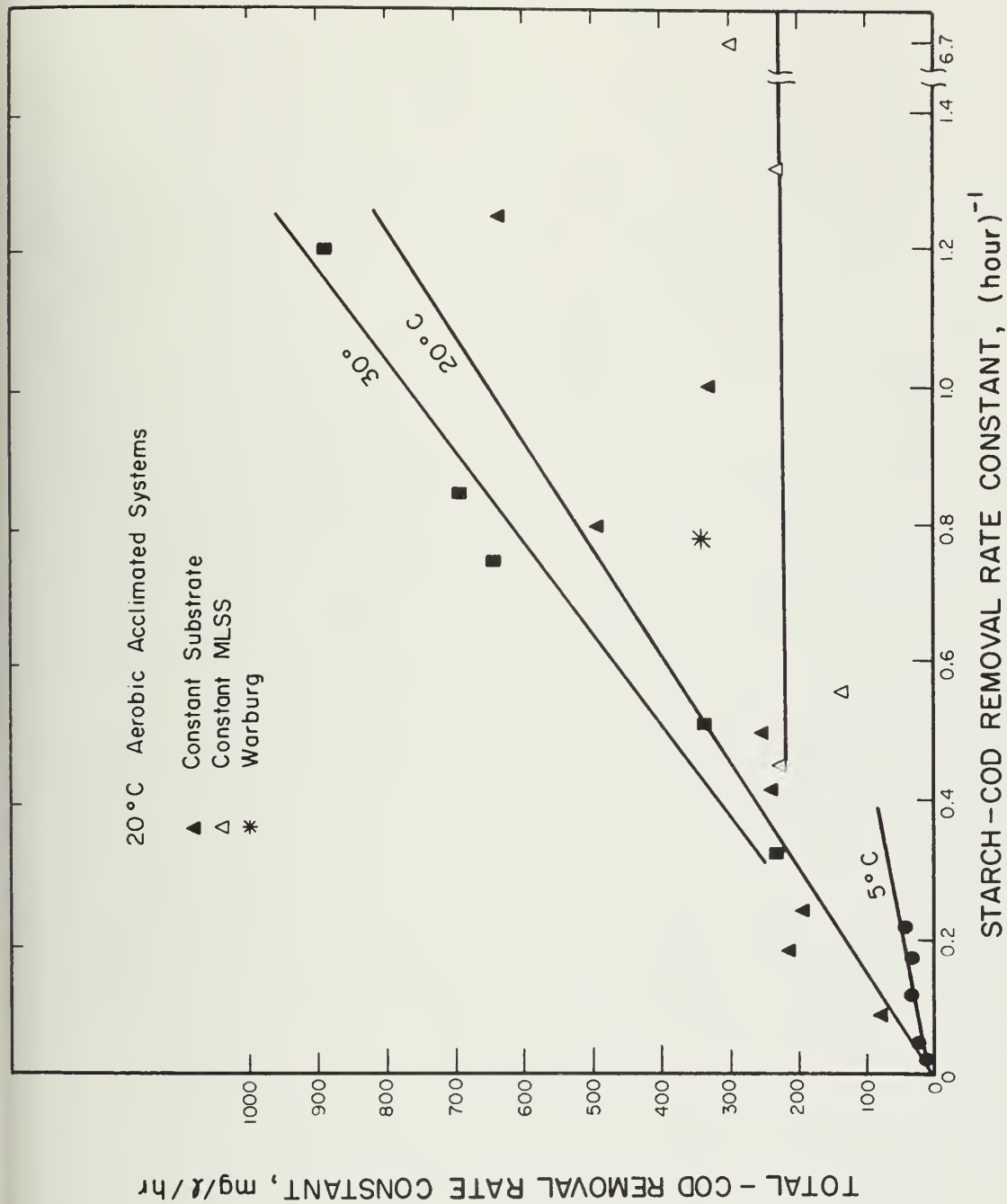


FIGURE 53: RELATIONSHIP BETWEEN RATE CONSTANTS OF STARCH - COD REMOVAL AND TOTAL - COD REMOVAL IN ACCLIMATED SYSTEMS

The initial velocities v were obtained from the initial slope of the starch-COD removal curves in Figures 28 through 31. Figure 54 shows the Michaelis-Menten plot of the data with constant sludge concentration system. These data (full circle in the Figure 54) did not produce the predicted straight line. This would perhaps indicate that the assumption involved in the Michaelis-Menten relationship (low enzyme concentration, rate limiting step, etc.) was not applicable in this case. Further, as has been pointed out the starch breakdown by the bacterial cell would be a very complex reaction and a single Michaelis-Menten relationship not capable of representing the kinetics. There was indication of inhibition of the initial starch-COD removal rate at the higher substrate concentrations. The starch-COD removal rate, as has been pointed out earlier, was dependent on F/M ratio, and so at higher substrate concentrations the amounts of enzymes present were in line with the relationship as shown in Figure 51.

The values of initial velocity v for constant substrate level but different sludge concentrations are also plotted on Figure 54. The variation in $\frac{1}{v}$ was due to the change in sludge concentration, since in Equation 8 $\frac{dL}{dt}$ depends also on sludge concentration. With the help of these constant substrate velocity of starch-COD removal data and the previously plotted constant sludge starch-COD removal rates, a straight line plot was made in Figure 54. The calculated V , the maximum initial velocity was 2100 mg/l/hr at the sludge concentration of about 800 mg/l. The L_K , obtained from the slope of the straight line, was 122 mg/l, which is the substrate concentration at half maximum velocity. However, the usefulness of this L_K and V value is doubtful since it was evident earlier that the data on the starch removal rate at constant sludge concentrations did not follow the Michaelis-Menten relationship. The straight line plotted in

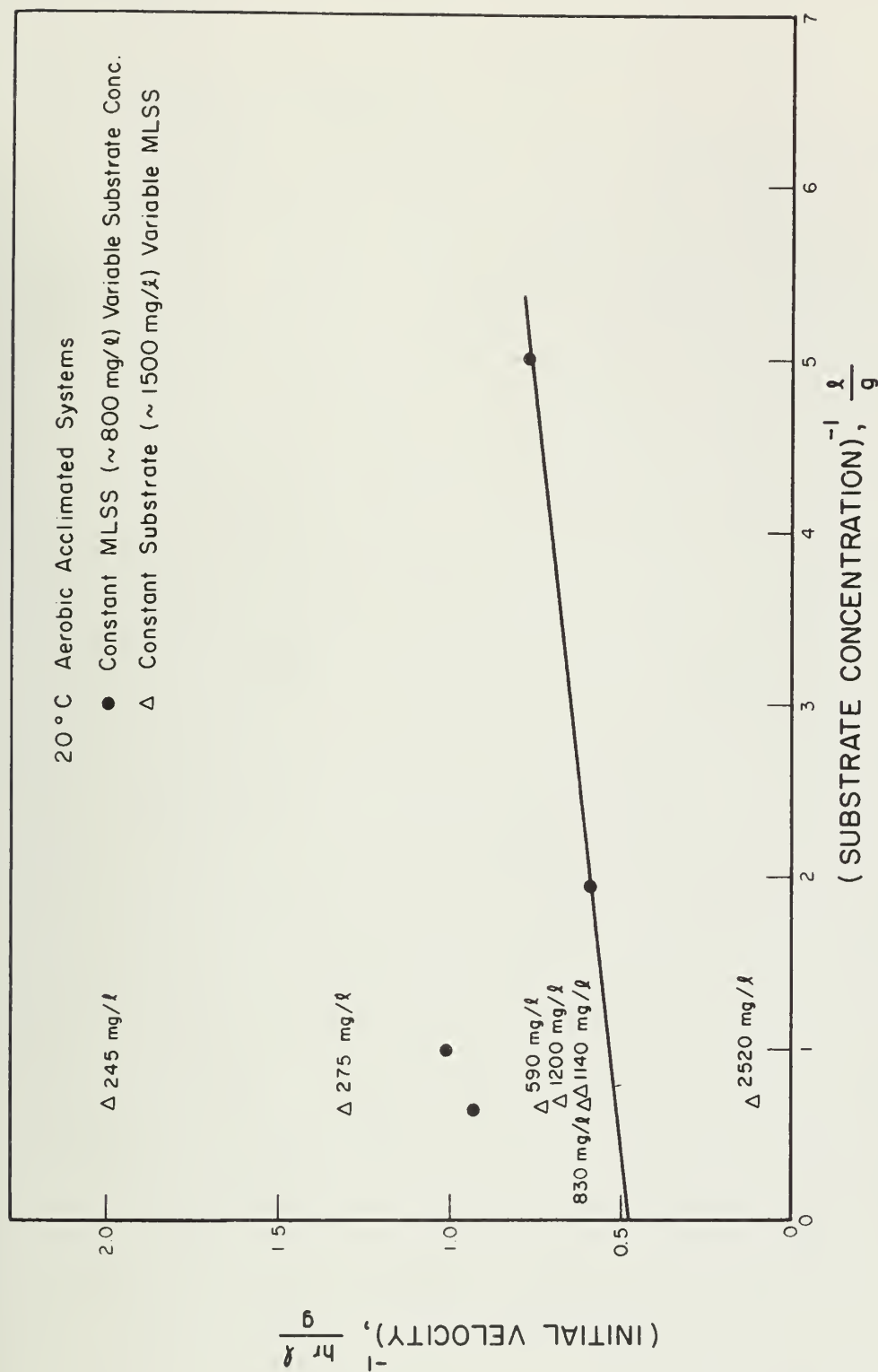


FIGURE 54 : MICHAELIS-MENTEN PLOT FOR STARCH-COD REMOVAL RATES

Figure 54 was perhaps not representative of the true situation.

7.1.2 Effect of Temperature

The effect of temperature on the starch-COD and total-COD rate constants has been shown in Figures 49 through 52. Table 10 represents the change in rate constants with temperature obtained from Figures 50 and 51 for the linear portion of the curves. If the temperature variation of the rate constants followed Van't Hoff-Arrhenius equation law, then a plot of $\log k$ vs. $\frac{1}{T}$ (k = rate constant, T = absolute temperature) would give a straight line. Figures 55 and 56 show the relationship between $\frac{1}{T}$ and the rate constants of total- and starch-COD respectively. The ΔE value in Equation 11 was calculated to be 21,000 cal per mole and 13,950 cal per mole for total-COD and starch-COD removal rates respectively. The ΔE value calculated for starch-COD removal rate was close to that reported for activated sludge systems of 14,400 cal per mole (34). The effect of temperature on "b" and "c" values, which were reported in Table 9 and which perhaps are more representative of the effect of temperature on the reaction rates since they eliminate a variable on which the rate constants depend, the initial solids in the case of total-COD removal and $(F/M)^{-1}$ for starch-COD removal, was determined. Figure 57 shows the relationship of temperature and these constants "b" and "c." The ΔE values calculated from Figure 57 were 20,400 and 16,500 cal per mole for the total-COD removal rate constant and the starch-COD removal rate constant. These values are slightly different from those obtained earlier. However, these values would be more representative of the actual situations since "b" and "c" are true constants at a fixed temperature, as compared to k values

TABLE 10
EFFECT OF TEMPERATURE ON STARCH REMOVAL RATES OF ACCLIMATED ACTIVATED SLUDGE SYSTEMS

Initial MLSS mg/l	Total-COD Removal Rates,			Starch-COD Removal Rates,			
	5°C	20°C	30°C	(F/M) ⁻¹	5°C	20°C	30°C
		mg/l/hr		mg/mg		(hr) ⁻¹	
400	12	160	285	0.3	0.062	0.30	0.43
500	15	190	345	0.4	0.077	0.40	0.54
600	19	222	400	0.5	0.092	0.50	0.65
700	21	252	460	0.55	0.100	0.56	0.71
800	23	282	515	0.60	0.105	0.61	0.82
900	26	314	575				
1000	29	344	630				

TOTAL-COD REMOVAL RATE CONSTANTS, mg/l/hr

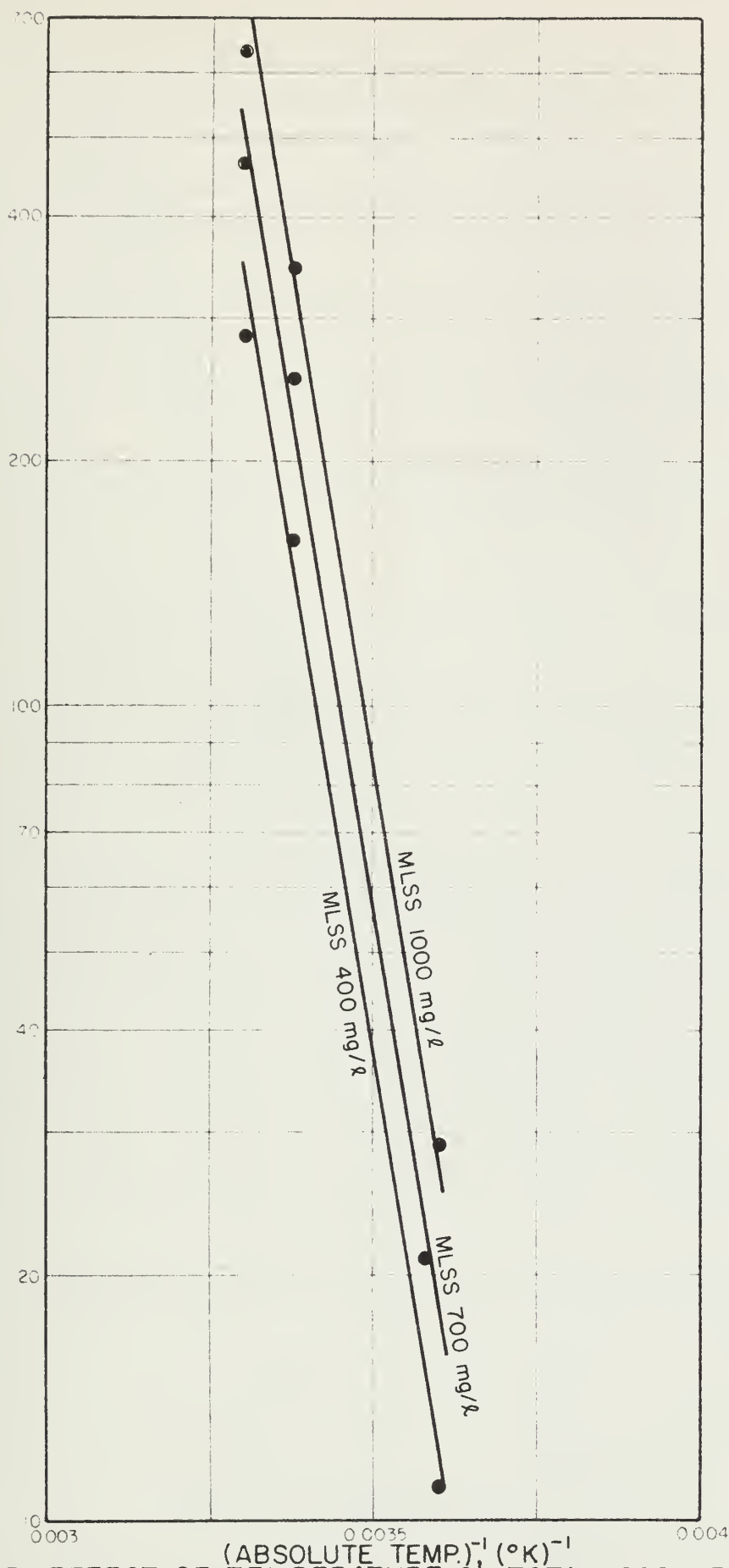


FIGURE 55: EFFECT OF TEMPERATURE ON TOTAL-COD REMOVAL RATE CONSTANTS

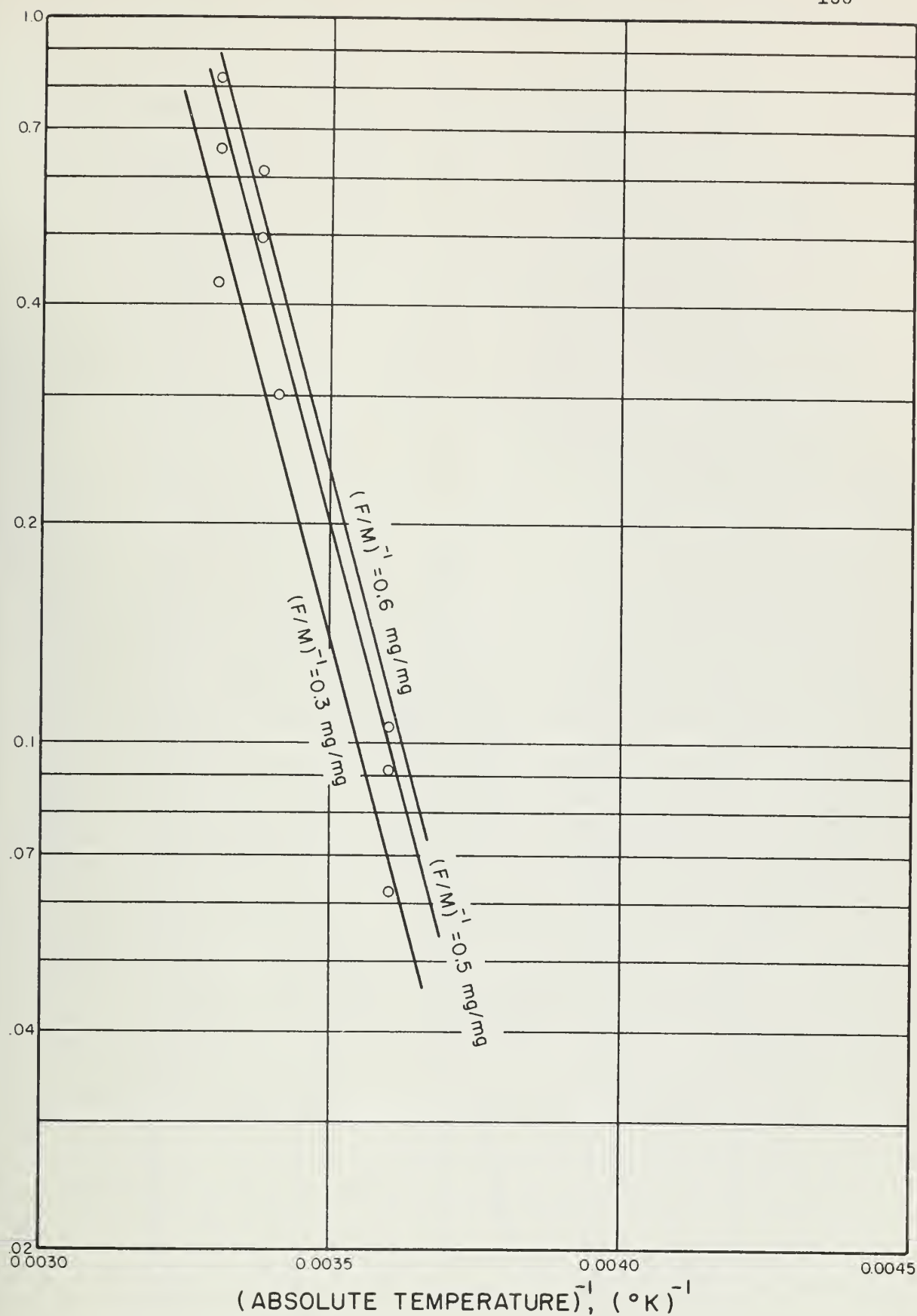
STARCH-COD REMOVAL RATE CONSTANT, $(\text{hr})^{-1}$ 

FIGURE 56 : EFFECT OF TEMPERATURE ON STARCH-COD REMOVAL RATE CONSTANTS

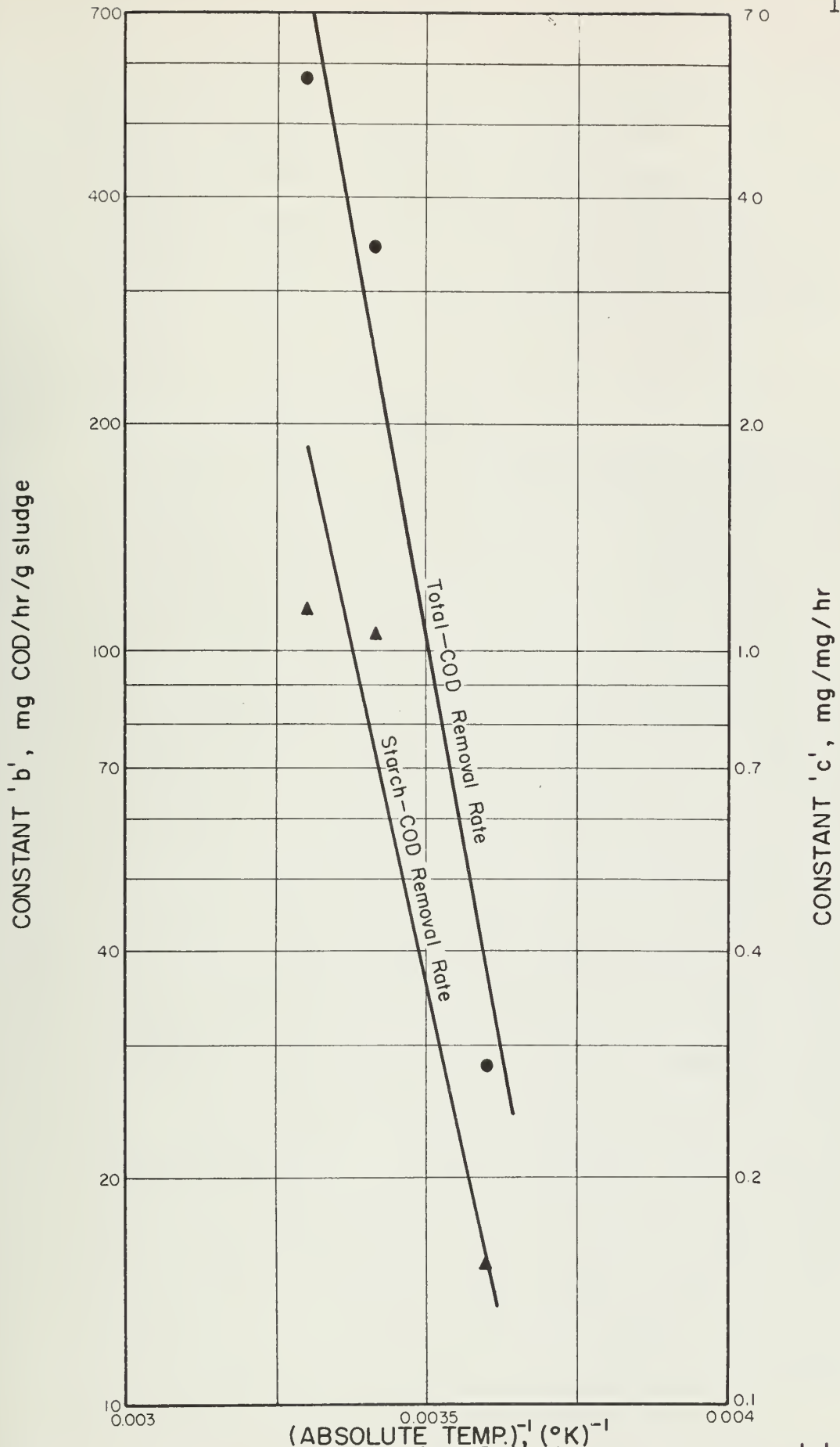


FIGURE 57 : EFFECT OF TEMPERATURE ON THE CONSTANTS 'b' & 'c'

which were shown to be dependent on initial solids concentration of the system in the case of the total-COD removal rate and $(F/M)^{-1}$ for the starch-COD removal rate. The calculated Q_{10} values between 20° and 30°C obtained from Figure 57 were 2.57 and 3.19 for starch-COD removal rate coefficient "c" and total-COD removal rate coefficient "b" respectively.

7.1.3 Effect of Oxygen Deficiency

The oxygen deficient studies involved blowing nitrogen gas in place of air through the glass diffuser in the acclimated activated sludge system at 20°C. The oxygen deficient system has been referred to as anaerobic from time to time, on the basis of the absence of aeration. Figures 33 and 34 indicate that the starch-COD was removed quite efficiently with the nitrogen-blown system, but total-COD remained practically constant. There was a slight decrease in the total carbohydrate content, indicating conversion of carbohydrate degradation product of starch to some other unidentified forms. The sludge concentration was also quite unchanged during the period of the test. These results would mean that the enzyme systems responsible for starch degradation were active under anaerobic conditions as long as there was good mixing of the substrate with the cells. The initial adsorption of starch by the sludge was also not affected by anaerobic conditions. The total-COD of the system remained constant at the level after adsorption even after all the starch was degraded. Thus, the amount of starch adsorbed was somehow utilized by the sludge, because the increased sludge mass obtained initially was maintained even after all the starch was degraded in the system. If the adsorbed starch was degraded and not utilized then we would have obtained a decrease in sludge mass, and an increase of the total-COD of the system to the value originally fed.

It is proposed that in the mixed population of the activated sludge system, a small number of microorganisms was capable of utilizing the starch substrate under anaerobic conditions. If the period of test was continued for longer periods, perhaps these microorganisms after multiplication and growth would have utilized all the total-COD remaining in the medium. This would be a typical case of selection or acclimation of the sludge to new anaerobic conditions. The starch-COD removal rate constant under anaerobic conditions is plotted in Figure 51. The rate constant was higher than under aerobic conditions at F/M 1.28, but was of the same order of magnitude as in the aerobic system at F/M 2.2. The reason for the high starch-COD removal rate constant at F/M 1.28 for anaerobic system as compared to aerobic system is not evident. There was a possibility that active cell mass in the former system was higher, but this explanation can only be hypothesized, since no measurements of active cell mass were made.

7.1.4 Cell-Free Starch Removal

The degradation of starch in the acclimated activated sludge membrane filtrate (referred to here as cell-free starch degradation), after a short period of contact between starch and sludge at different temperatures and growth conditions, has been presented in Figures 5, 8 and 9 for 5°C, Figures 11, 12, 28, 29, 30 and 31 for 20°C, and Figures 22, 23, 24, 25 and 26 for 30°C. The cell-free starch-COD removal rates were, in each case, much lower than the rates obtained with whole cells, and for the period of test followed linear removal kinetics. The cell-free starch-COD removal rates have been reported in Tables 4, 5 and 6 for the three different temperatures. There was some variation of the cell-free starch-COD removal rate with different F/M ratios at a particular temperature, but on the

whole, the rates increased with an increase in temperature. The cell-free starch-COD removal rates were only 0.2 to 9 percent of the initial whole-cell starch-COD removal rate. (The average value was about 2 percent.) Thus, it would seem that starch degradation by acclimated sludge filtrates (cell-free) was negligible as compared to whole-cell starch degradation. It would be concluded from the above that the amount of exoenzymes secreted by the cells in the medium was insignificant. It has been reported by various investigators (74)(77)(78) that amylases of most microorganisms were liberated in the medium (extracellular) and in many cases had been isolated from culture filtrates (79)(80). These reports were contrary to the findings here. It may be argued that this discrepancy may be due to three reasons, (i) there was inhibition of amylase by high substrate (starch) concentrations, (ii) the use of phosphate buffer removed the calcium ions necessary for amylase activation, or (iii) the enzyme system responsible for starch degradation was situated on the cell wall and not liberated in the medium under conditions of the test.

In the studies, starch concentration had been about 1500 mg/l. In Figure 12 the cell-free starch-COD degradation was determined at different times of contact between starch and acclimated sludge; i.e., 5 minutes and 20 minutes. At the start of each of the cell-free starch-COD degradation tests, the starch concentration was different. At 5 minutes the starch concentration in the medium was around 1000 mg/l and at 20 minutes it was about 600 mg/l. Even so, the rate of degradation of starch was the same for both cases (16 mg/l/hr).

In another study the concentration of sludge was maintained constant and the concentration of starch was varied from 200 mg/l to 1525 mg/l (Figures 28 through 31). There was hardly any inhibitory effect

of high starch concentration noted in the cell-free starch-COD degradation rate. As a matter of fact, there was an increased cell-free starch-COD degradation activity at higher substrate concentration, as reported in Table 5, although still the cell-free rates were much lower than the whole-cell starch degradation rates. Nomura et al. (81) found that increased starch concentrations produced higher amylase yields in B. subtilis. Thus, it was unlikely that high starch concentrations were inhibitory for enzymes in sludge filtrates. Further, there was no indication of inhibition by high substrate concentration in the whole-cell starch-COD degradation, so the high substrate inhibition of amylase in sludge filtrate was ruled out.

The second possibility of low cell-free amylase activity due to the calcium ion removal by phosphate buffer used, has been reported for Malt- α -amylase (73). However, in bacterial amylases the calcium ion is so thoroughly complexed with the enzyme that extra addition of calcium ion for activation is not necessary (76). Further, Fukumoto et al. (82) reported enhanced amylase production by B. subtilis with phosphates, especially when carbohydrates were used as carbon source. The experiment with acetate buffer, Figure 35, shows that the starch-COD removal rate with whole-cell system was very similar to that obtained with phosphate buffer at comparable F/M and temperature. However, there was a stimulatory effect of acetate buffer on cell-free starch degradation (Table 5) as compared to systems with phosphate buffer. The cell-free starch-COD degradation rate with acetate buffer was still only about 4.5 percent of the initial starch-COD removal rate with whole cells. Thus, although the results of acetate buffer system did not answer the question of low starch degradation activity of cell-free sludge filtrates, there was a higher activity of amylase in the

filtrate compared to phosphate systems. It was pointed out by Fukumoto et al. (82) that the earlier the phosphate was added to B. subtilis system the larger was the amount of amylase produced, but the amount of the protein secreted to the medium was lower. So the decreased amylase activity in cell-free sludge filtrates with phosphate buffer compared to acetate buffer systems could be due to lower enzyme secretion from cells to the medium in the phosphate system, although the total enzyme activity with whole cells was similar in both cases. Thus, the stimulatory effect of acetate buffer compared to phosphate buffer for higher amylase activity in sludge filtrates was perhaps not due to calcium ion removal in case of phosphate buffer but due to reduced amylase secretion into the medium by the cells in a phosphate buffer system.

The third possibility for the low amylolytic enzyme activity in sludge filtrate was the presence of a large fraction of the starch degrading enzyme system in association with the cell walls. These enzymes are attached to cells and degrade starch on the cell surface rather than in the medium. Only a small fraction of the total amylase is secreted in the medium producing low starch degrading activity of the cell-free sludge filtrates.

In order to determine if the cell walls had high amylase activity, acclimated sludge cells were disrupted. The isolated crude cell wall fractions were separated from the soluble cell components and its starch-COD degrading activity plotted in Figures 47 and 48. It will be evident from Figure 47 and Table 8 that the starch-COD degrading rate for the cell-wall fraction was much higher than even whole cells when expressed on the basis of total protein of the system. Although the techniques for isolation of cell walls were not very refined, the results show very definite indications

that the amylase system for the sludge was located in association with the cell walls. The higher rate of starch-COD degradation with cell wall preparation was perhaps due to higher enzyme amount per gram of cell wall as compared to whole-cell.

In Aspergillus oryzae, α -amylase has been known to be bound to the cell wall depending on the pH of the medium (83). Thus, it would be possible to have a system in which the microenvironment of the cell has a pH different from the medium which allowed retention of α -amylase on the cell surface. Most of the reports (77)(78) indicate that in bacterial systems the location of amylase is extracellular. Recently there have been some reports of bacterial intracellular amylases (84)(85), which could be the precursors of the extracellular amylases. However, the location of amylase on the surface of the cell has not been reported in any bacterial system.

The high starch degrading activity (Table 8) of the soluble cell component was perhaps due to the presence of intracellular amylase or phosphorylase. The presence of intracellular amylase in some systems has been proved beyond doubt (85). In S. bovis cell extract Ushijima and McBee (86) reported the presence of a phosphorylase capable of degrading starch. No reports of extracellular phosphorylase have been made and all phosphorylase systems are perhaps intracellular. Therefore, the phosphorylase could also be present inside the cell, and no attempts have been made to characterize the exact nature of the enzymatic system in the soluble cell component responsible for the degradation of starch.

The finding of Nomura et al. (74)(87), that extracellular amylase production in B. subtilis was low in the log growth phase but high in the stationary growth and autolytic phase, was also tested. Figure 36 and

Table 5 show that sludge filtrates in the endogenous phase had a much higher starch-COD removal rate compared to sludge filtrates in the log growth phase. But even in the case of endogenous sludge filtrates the starch-COD removal rate was much lower than with whole cells. In the studies with endogenous sludge filtrates no measurements were made to see the amount of autolysis of cells, so the higher activity of enzymes could be due to liberation of intracellular enzymes or surface bound enzymes by autolysis or changed physiological state of the cells.

From the preceding discussion it would be fair to infer that the major part of the enzyme system responsible for starch degradation (amylases) was situated on the cell surface with minor amounts liberated in the medium.

7.1.5 Synthesis of Sludge

In the acclimated systems studied, the total amount of sludge synthesis from the starch substrate fed in cases where the total-COD was completely utilized after six hours of aeration (except the residual stable organic end product COD) varied from an average of 64 to 59 percent of the total-COD fed at 20°C and 30°C respectively for constant substrate level systems. For the system with constant sludge concentration, the percent of substrate concentration converted to sludge mass was 58. The values are referred to as "a" value in Equation 2. Placak and Ruchhoft (9) and Sawyer (88) have reported similar values of sludge synthesis with carbohydrate substrate.

7.2 Starch Removal in Non-Acclimated Systems

In these experiments there were three classes of organic compounds to which activated sludge from the basic seed unit was acclimated. The first was carbohydrates which constitute monomers for the starch molecule; i.e., glucose and maltose, the second class of compounds was a complex compound Metrecal, and the third class was a protein; i.e., bovine serum albumin.

7.2.1 Starch Removal by Glucose and Maltose Systems

As has been mentioned earlier, starch on enzymatic degradation may produce substantial quantities of glucose and maltose molecules besides other oligosaccharides. Further, in order to utilize maltose as a substrate compared to glucose, a cell would have an extra enzyme, the maltase. It has been reported that maltose and maltodextrins have a stimulatory effect on α -amylase formation in B. stearothermophilus (89), whereas glucose had no such effect. The amylase formation of B. subtilis is influenced by the carbon source but in the presence of casein as nitrogen source, most sugars are available as carbon source (82). However, glucose at high concentration is inhibitory to amylase formation in B. subtilis (78), at low concentration it is available for enzyme formation. Fukumoto et al. (78) concluded that different carbon sources in the production of amylases from B. subtilis merely served as energy suppliers, and the observed divergence of the effect of carbon source on amylase formation was due to the competition between the amylase forming systems and other enzyme systems of the cell for the same nitrogen compounds.

The degradation rate of starch in maltose system at F/M ratios

1.2 and 2.9 are presented in Figures 40 and 41. The degradation rate was slower than a comparable starch acclimated system but there was no lag observed in either case. This clearly indicates that the amylase system of maltose acclimated sludge was present even at the start; i.e., the enzyme was constitutive. However, the lower rate of starch and total-COD reduction indicate that the activity or amount of enzyme present under the conditions was lower than the comparable starch-acclimated system. The total-COD removal characteristic in Figure 40 was slightly different than observed in the starch-acclimated system. After a linear total-COD removal the first two hours, the rate suddenly changed to another linear rate.

(This peculiarity was not observed at the higher F/M in Figure 41.)

Apparently the starch-COD removal rate was unaffected by this sudden change in the total-COD removal rate. Thus, the elimination rate of starch degradation products was altered, not the starch degradation itself. The high rate of build-up of some starch degradation products was inhibitory to the cell at some stage involving their assimilation.

Figures 37 and 38 represent the starch removal characteristics of glucose acclimated sludge. In Figure 37 (F/M 1.24) the starch-COD and total-COD removal rates did not have any lag and the removal characteristics were very similar to those of the starch acclimated systems, although the removal rates were lower. However, in Figure 38 (F/M 2.54), the total-COD removal rate was retarded initially, but the starch-COD removal proceeded at a slow rate without any lag. The assimilation of the starch degradation product in this system was retarded. This is attributed to the low concentration of the enzymes responsible for assimilation of the starch degradation product (consequently the removal of total-COD); the time required for the induction of more enzymes caused the lag in the total-COD removal.

The total-COD and starch-COD removal rates of glucose acclimated sludges were lower than the comparable maltose or starch acclimated systems (Figure 39, Table 5 and Table 7), but there was no lag involved in the starch utilization. This would indicate that the enzymes responsible for removal were constitutive in the glucose systems, although the total amount of the enzymes produced initially was less than that in the maltose or starch acclimated systems.

The starch removal rate by the glucose sludges was lower than that of maltose sludges. This may be expected, since the glucose sludge may have to evolve or reactivate in quantities at least two enzymes, amylases and maltases, before the starch can be utilized. In maltose systems it may be possible to get by with the evolution or reactivation of only one enzyme, amylases, for the utilization of starch. Thus, maltose sludges can be expected to degrade starch at a faster rate initially.

An attempt was also made to see the change in starch removal rate when a glucose acclimated sludge was gradually becoming acclimated to starch. Figures 44, 45 and 46 show that within 4 days the rate of total-COD and starch-COD was of the same order of magnitude as starch-acclimated systems (Table 5). This change in starch removal rates was brought about either by increased induced enzyme production by the starch substrate or by change in predominant microorganisms most suitable for utilizing starch as substrate. It will be difficult to pinpoint what was actually happening from the data obtained. However, from the point of engineering considerations, it is evident that glucose sludges can be completely acclimated to starch substrate in a relatively short time. Thus, a qualitative shock load of this kind in an activated sludge system can be taken care of in a matter of days.

7.2.2 Starch Removal by Metrecal System

The Metrecal substrate is a mixture of various natural ingredients, each quite complex in chemical composition. Table 1 lists a few of the properties. It will be evident that Metrecal has about 11.6 percent carbohydrate and the manufacturer even mentions the presence of starch on the packaged product but information about the amount of starch is not made available. Thus, the feeding of starch substrate to Metrecal-acclimated sludge was a partial shock load since microorganisms in the sludge were familiar with such compounds.

The starch removal characteristic of Metrecal-acclimated sludge has been presented in Figure 42. There was a long lag period in the total-COD removal, whereas starch-COD removal rate was very slow but did not indicate an appreciable lag. This would indicate that perhaps the cell assimilative system for the starch degradation products was not developed until the end of the lag period. In the Metrecal system, too, the amylolytic enzymes seemed to be constitutive since there was no appreciable lag in the starch-COD removal rates. The starch removal rates when compared with maltose and glucose acclimated sludge were generally lower for the Metrecal system (Table 7).

7.2.3 Starch Removal by Bovine Serum Albumin System

This substrate is chemically very different from the starch molecule, so it would be expected that there would be a long lag before any degradation of starch by this system occurs.

In Figure 43 it will be seen that there was quite a marked lag in both starch-COD and total-COD removal. The total-COD removal rate was

lower than the acclimated system even in the linear removal phase following the lag. The enzyme system responsible for starch removal with bovine serum albumin acclimated sludge was adaptive. The presence of starch induced the amylolytic enzymes in the sludge after a period of lag. This shows that the enzyme systems responsible for starch degradation are quite easily inducible even with sludges acclimated to quite different structural compounds.

7.3. Adsorption of Starch Substrate by Sludges

In all the systems using starch substrate, especially with starch acclimated systems, it was reported that there was immediate removal of substrate on contact with sludge. This initial removal was referred to as adsorption. The removal of substrate from the system by sludge was invariably indicated by a comparable increase in the weight of sludge. The possibility of the substrate being removed on the filter paper and giving a false initial adsorption phenomenon was eliminated by use of substrate previously filtered through a similar membrane filter. Thus, any immediate removal of the starch substrate by the sludge was attributed to adsorption.

7.3.1 Adsorption by Acclimated Systems

The amount of adsorption of the starch by the acclimated sludge at different temperatures has been presented in Tables 4, 5 and 6. The concentration of initial substrate, after adsorption had occurred, is also presented. There was generally higher amount of starch adsorption at 5°C than at 20°C under comparable situations. But the adsorptions at 30°C were generally higher than both 20°C and 5°C. Thus, the generalization that low

temperature favors higher adsorption was not wholly applicable here. Attempts to determine the amount of starch adsorbed at different times after initial contact were not successful, hence it was not possible to determine the equilibrium concentration. However, it must be remembered that adsorption of starch to bacterial cells which degrade it would produce a complex dynamic equilibrium. It would be very difficult to theoretically evaluate such a system where adsorption, degradation and growth are going on simultaneously. Attempts were made to plot $\log x/m$ versus initial substrate concentration for the adsorption data at the three temperatures, according to Equation 13a. The great scatter of points in Figure 58 indicates that simple assumptions made in Freundlich's derivation are not valid when the starch substrate is adsorbed on bacterial cells. The data plotted in Figure 58 were not obtained under equilibrium conditions, and it was doubtful if such equilibrium conditions did exist in the experimental system at any time.

The adsorption data of 5°C, where the metabolic activity of sludge would be low, were plotted in Figure 59 to see if adsorption increased with increased sludge concentration. Meanwhile the starch concentration was kept constant. There was a linear relationship in Figure 59, indicating a mass effect rather than an equilibrium situation based on adsorption and desorption.

The adsorption of starch on sludge under anaerobic conditions, Figures 33 and 34, was of the same order of magnitude as with the sludge under aerobic conditions. This would mean that even in conditions of no growth the adsorption was present. Adsorption would thus seem a physical process of removal of starch by sludges.

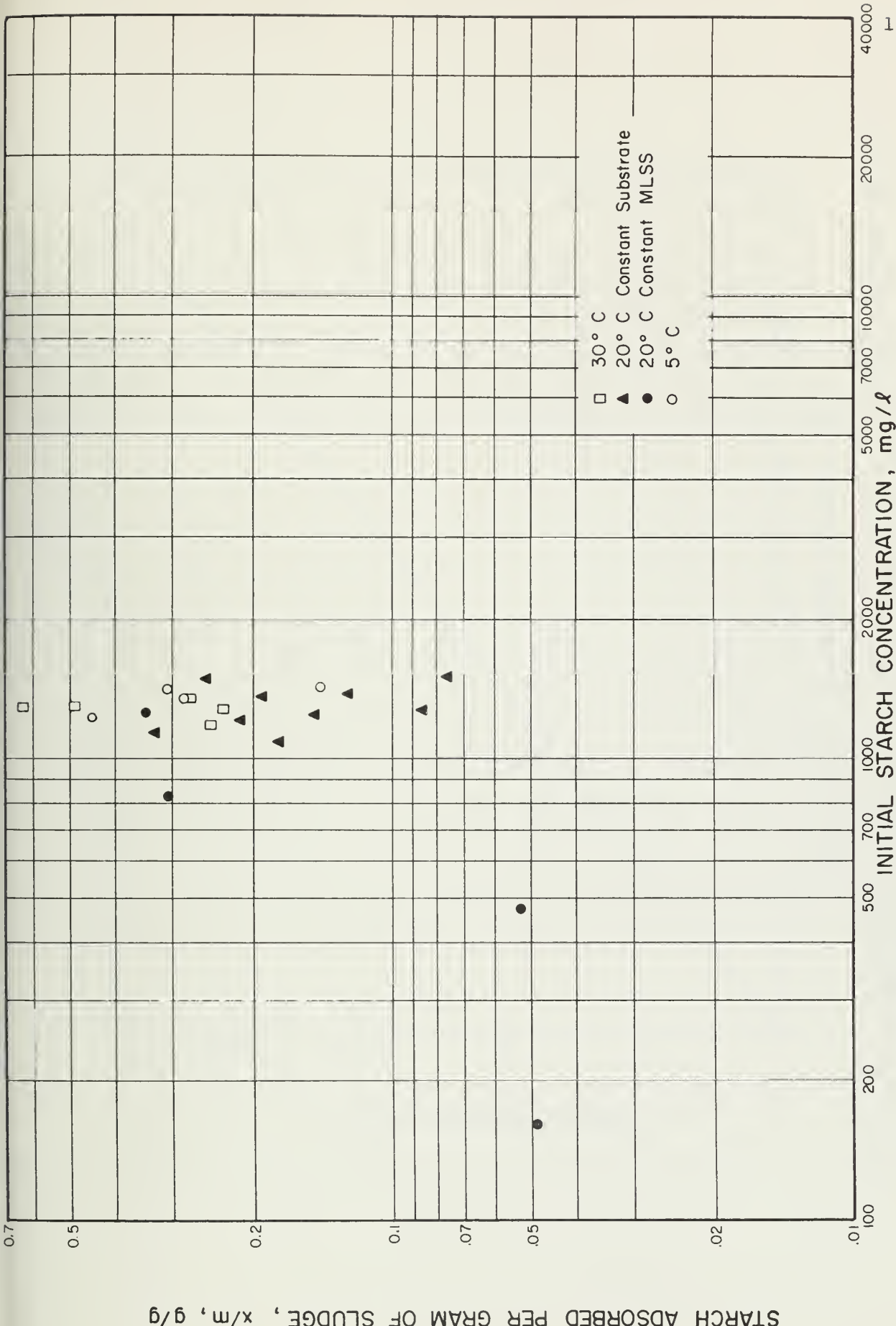


FIGURE 58: ADSORPTION OF STARCH BY THE ACCLIMATED SLUDGE SYSTEMS

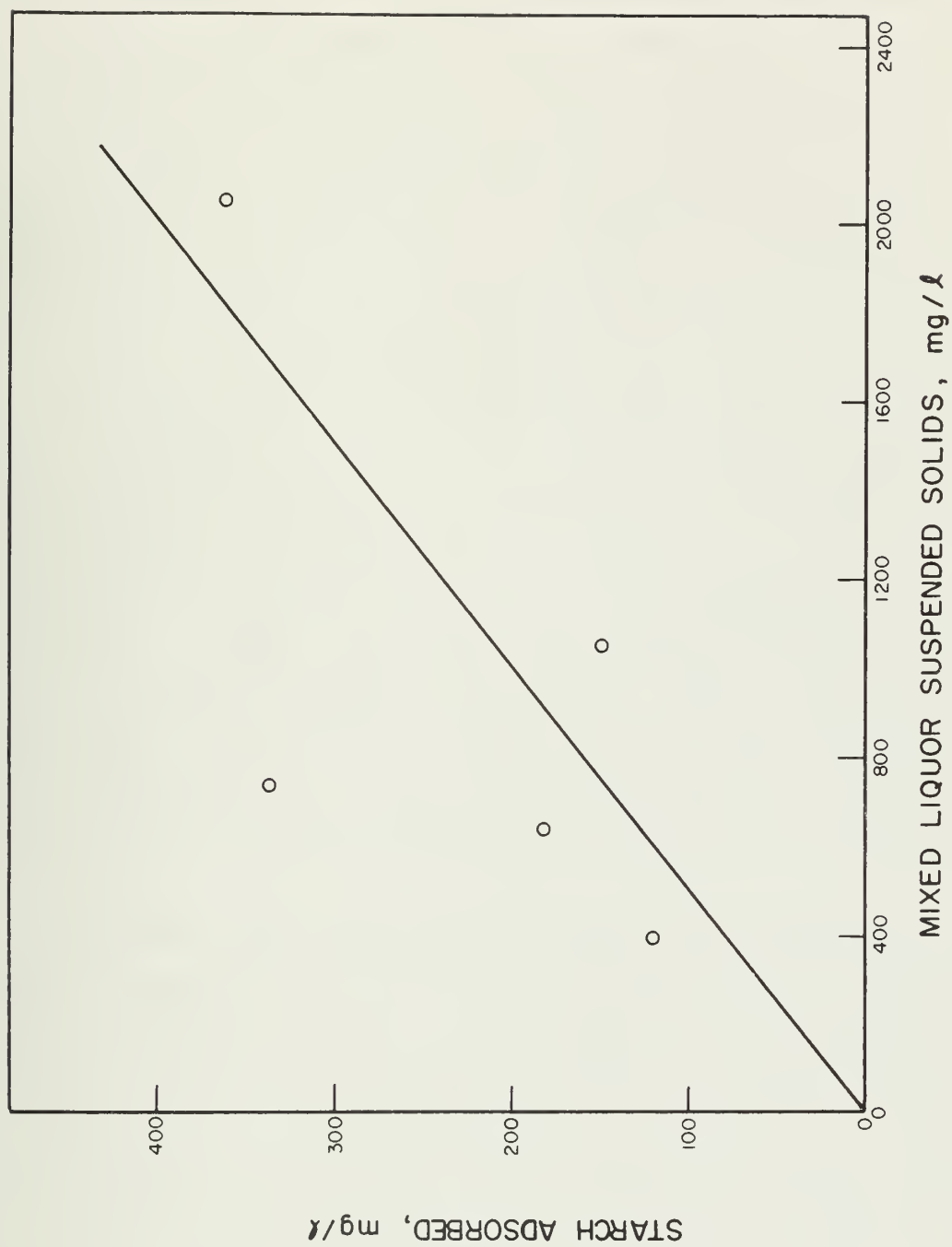


FIGURE 59 : ADSORPTION OF STARCH BY ACCLIMATED SLUDGE SYSTEMS WITH DIFFERENT SLUDGE CONCENTRATIONS AT 5°C

7.3.2 Adsorption by Non-Acclimated Systems

The amount of starch adsorbed in almost all non-acclimated systems was very much lower than the acclimated systems under similar F/M conditions. This is evident by comparing initial starch adsorption data in Table 5 and Table 7. Thus it would appear that adsorption of starch on activated sludge was also dependent on the acclimation of the sludge. This was further demonstrated in the Figures 45 and 46. The change in starch substrate removal characteristics was determined with glucose acclimated sludge, over a period where the sludge became finally acclimated to starch substrate. The change in initial adsorption with succeeding days was plotted in Figure 60. There was an immediate increase in adsorption of starch substrate by the glucose sludge as it was getting acclimated to starch substrate. However, after getting completely acclimated, there was a drop of initial starch adsorbed on the 6th day. This was very unexpected and unexplainable. But it would seem that adsorption of starch on the sludge was dependent on the acclimation characteristics of the sludge to starch.

So, on the whole, adsorption of starch on sludge constitutes a valid means of its removal but the exact amount would depend on many factors like temperature, amount of sludge, acclimation and physiological condition of the sludge. The influence of acclimation on the amount of starch adsorbed would indicate a more complex situation than a mere physical phenomenon. It would be tempting to hypothesize that increased adsorption of substrate on cells after acclimation was facilitated by cellular enzyme systems on the surface of the cell, since it has been found that in our case the amylolytic enzyme systems of starch acclimated sludges were located on the cell surface. However, no proof of such a hypothesis is available at

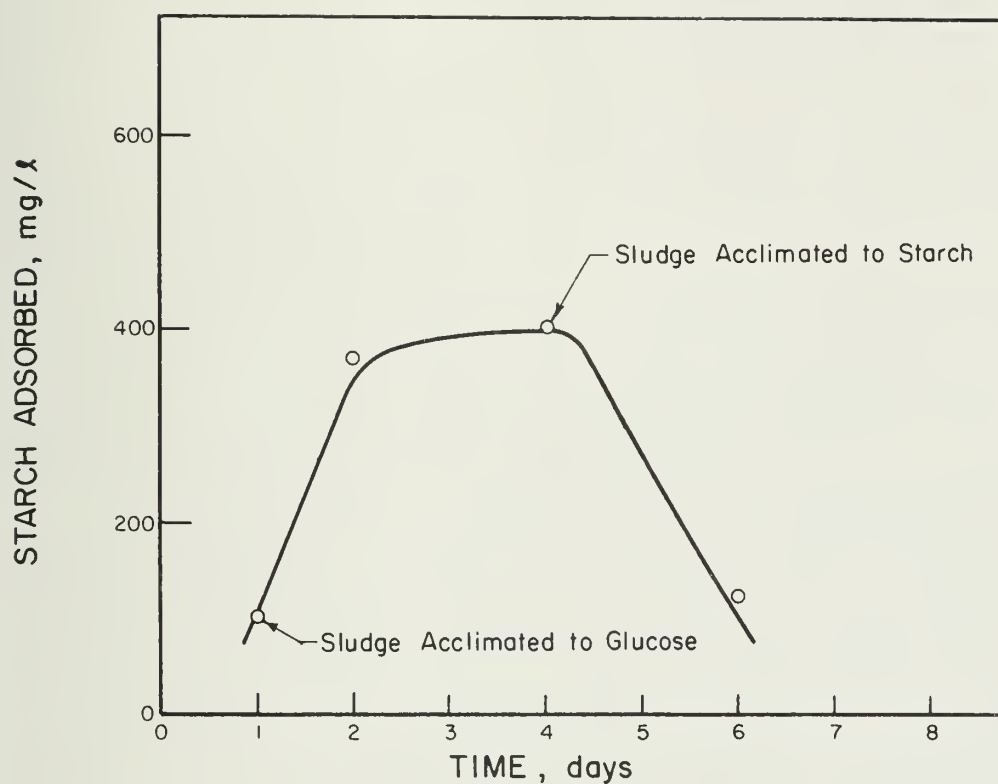


FIGURE 60: CHANGE IN STARCH ADSORPTION BY GLUCOSE SLUDGE WITH ACCLIMATION TO STARCH

the present moment. The removal of starch from the medium was due to two processes, adsorption of starch on the cells followed by its enzymatic degradation on the cell wall. The high positive values of Q_{10} , the temperature coefficient for the starch-COD removal, indicated that the rate controlling reaction was enzymatic reaction rather than physical process. The effect of temperature on physical processes like adsorption is usually inverse, i.e., increase of temperature decreases the adsorption. However, there still is a possibility that the adsorption of starch on the cell surface was occurring at sites where the exoenzymes were situated. In this case the high positive value of Q_{10} would indicate the total effect of two enzymatic processes.

There is also a possibility of formation of "active sites" for starch adsorption after acclimation of a particular sludge. However, here also no proof of existence of such sites is available.

The average size of a starch molecule in solution was not available at the present time. However, the molecular dimensions of glycogen, a polysaccharide of similar structure as amylopectin fraction of starch, has been reported as 15 μ in diameter (90). The shape of the glycogen molecule is globular as opposed to the linear shape of starch molecules. If the size of starch molecules is assumed to be the same as that of glycogen, then approximate calculation can be made to see what amount of starch can be adsorbed per gram of sludge if monomolecular coverage of the cell surface is assumed.

Projected area of starch molecule = 175 μ^2

Surface area of 1 μ diameter cocci cells = $3.14 \times 10^6 \mu^2$

Number of molecules of starch in a monomolecular

$$\text{coverage of the cell} = \frac{3.14 \times 10^6}{175} = 18000$$

From Table 2 the average molecular weight of potato starch, assuming 23 percent amylose and 77 percent amylopectin, was 185,000. Therefore 1 molecule of starch would weigh 3.1×10^{-16} mg and the amount of monomolecular starch adsorbed per cell = $18000 \times 3.1 \times 10^{-16}$ mg = 5.6×10^{-12} mg. If the weight of a cell was assumed to be 1×10^{-12} g (91), then the amount of starch adsorbed on cells (monolayer adsorption) would be 5.6 mg/gram of cell. However, the experimental value of the starch adsorbed per gram of sludge at 20°C was on an average 150 mg. This discrepancy can be explained, either by the fact that multilayer starch adsorption was occurring on the cells or the linear starch molecule oriented in such a way had much smaller surface area of contact as compared to that assumed for globular glycogen.

From the calculations above it is evident that adsorption of starch on the cells covers at least a monolayer and the possibility of starch adsorption only on the specific sites where amylolytic enzymes were situated on the cell surface would be much less.

Therefore, it appears that adsorption of starch on sludge is a physical phenomenon. The acclimation of sludge to starch causing high starch adsorption is perhaps either due to a change in the predominant microbial population of the system, or due to a change in the surface characteristics of the cells.

In the field of microbiology, various references have been made in which macromolecular substrates are adsorbed and degraded by enzymes located on the surface, in confirmation to our findings with starch. The breakdown of cellulose by Cytophaga has been attributed to surface located enzymes (92) and adsorption of the cells on cellulose micelles was the predominant mechanism of degradation of cellulose.

The hydrolysis of ribonucleic acid by Pasteurella pestis has also been reported to be due to surface adsorption of the substrate and its subsequent degradation by the surface located enzyme (92).

In passing, mention may be made about the adsorption of some antiseptics, like phenols and esters of acids, to bacterial cells. It has been reported that the mechanism of toxicity in such cases is due to the adsorption (93). The random adsorption of bacteriophage to cells has already been mentioned earlier.

7.4 Mechanism of Starch Removal

In the systems investigated the mechanism of removal of starch has been postulated as follows:

(i) a portion of starch was adsorbed on the sludge, depending on the conditions (temperature, acclimation, amount of sludge, physiological condition of sludge).

(ii) the adsorbed starch was degraded by cell-wall associated enzyme systems into smaller degradation products.

(iii) the starch in the medium was either adsorbed on the cell sites vacated by degraded starch molecules, or was degraded by small amounts of extracellular enzyme system in the medium. The former was much more effective compared to the latter in starch removal.

(iv) the starch degradation products were then utilized by the cell for assimilation.

In non-acclimated systems induction or reactivation of enzyme system was required before any starch degradation occurred by the mechanism proposed in the above paragraph.

7.5 Engineering Significance

It has been shown here that the removal of a colloidal waste, like starch, follows linear kinetics very similar to those of many industrial wastes, like pharmaceutical, brewery and refinery wastes, which have high colloidal content. Even some simple molecules, like butyrate, glucose and aspartic acid, have linear removal kinetics under aerobic conditions (37), as was pointed out earlier. Thus, colloidal wastes may follow kinetics quite similar to those of simple compounds, and the design criteria governing the removal of simple compounds in activated sludge would be applicable quite appropriately to the removal of colloidal waste.

However, the removal mechanism of a colloidal waste like starch was found to be quite different from that of small molecules, like glucose, lactose, etc. In the case of removal of starch, initial adsorption of substrate on sludge was evident in almost all the cases studied. This phenomenon of adsorption has not been observed (10)(24) with simple small molecules, and high initial removal in these systems has been attributed to high rate of assimilation.

Therefore, the removal of colloidal wastes like starch would be very effective in the contact stabilization process. A contact time of a few minutes would, in some instances, be able to remove a very high amount of total-COD input if the MLSS is maintained at a fairly high level. The starch degradation would be proportional to F/M ratio but initial starch adsorption would be proportional to the initial MLSS. Further, the total-COD removal rate, indicative of assimilation of starch degradation product, would be proportional to initial MLSS too.

Further, in the case of shock loads involving such colloidal wastes to an activated sludge plant, the resulting upset situation may be overcome

by increasing the amount of return sludge, or in other words increasing the amount of MLSS in the system. The increased sludge concentration would have to be such that suitable F/M is maintained for starch degradation.

The starch degradation and adsorption was not affected by anaerobiosis. This would indicate that in a conventional activated sludge plant treating starch waste, the aeration of sludge-waste mixture at the head end of the tank would not be necessary. A small mechanical mixer would be sufficient to permit starch degradation, followed by aeration to assimilate the degradation products. In the case of contact stabilization process a short mixing chamber may be sufficient to allow high starch adsorption without the need of aeration. The amount of sludge carried in the contact tank under anaerobic conditions will not be limited by the oxygenation capacity of the aeration device in aerobic systems. Very high starch removal may be obtained with high MLSS (above 10,000 mg/l) in the contact tank and short contact period. Longer contact time would be harmful since the effluent would carry the starch degradation products.

It would be apparent from the Table 5 that in the acclimated activated sludge systems with starch as substrate, generally 150 mg/l of total-COD was adsorbed initially at a MLSS concentration of about 1000 mg/l. On extrapolation to a contact stabilization process treating starch waste equivalent to 1000 mg/l COD, a 5-10 minute contact time with 5000 mg/l mixed liquor suspended solids concentration would give a removal of 750-800 mg/l COD by adsorption. Longer contact periods may not be necessary since the starch removal by adsorption is practically instantaneous. The optimum contact periods and MLSS concentration in the contact tank would have to be determined by further experimentation.

It would be worthwhile to see if the data presented allows to determine by interpolation the starch removal at other operating conditions.

For example, what would be the starch removal characteristics at 12°C, initial mixed liquor suspended solids concentration of 1400 mg/l and starch-COD concentration of 1800 mg/l? From Figure 57 the "b" and "c" values for 12°C (285°K) was 95 mg/hour/g sludge and 0.330 mg/mg/hr respectively. Using Equation 15, the value of 135 mg/l/hr was obtained for k_t , the total-COD removal rate constant for initial MLSS of 1400 mg/l. Similarly the k_s , the starch-COD removal rate constant, was found to be $0.256 \text{ (hour)}^{-1}$ for F/M ratio of 1.285 from Equation 18. It would take about $\frac{1800}{135} = 13.4$ hours to completely remove the total-COD of 1800 mg/l. However, some 40-80 mg/l COD would still remain in the medium owing to stable metabolic end products. But this still would give a removal efficiency of above 95 percent. For 95 percent starch degradation it would take 5.1 hours if the Equation 17 is used, i.e., logarithmic degradation of starch-COD.

Table 11 gives the details of the above calculations.

TABLE 11

CALCULATION DETAILS FOR DETERMINING BY INTERPOLATION
THE STARCH DEGRADATION CHARACTERISTICS AT 12°C,
1400 mg/l MLSS AND STARCH-COD CONCENTRATION OF 1800 mg/l

From Figure 57: AT 12°C (285°K)

"b" = 95 mg/hr/g sludge and

"c" = 0.330 mg/mg/hr

From Equation 15: Total-COD removal rate constant $k_t =$

$$b(S_o), S_o = 1400 \text{ mg/l}$$

$$\therefore k_t = 1.40 \times 95 = 135 \text{ mg/l/hr}$$

From Equation 18: Starch-COD removal rate constant $k_s =$

$$c (F/M)^{-1}, (F/M)^{-1} = 0.778$$

$$\therefore k_s = 0.256 \text{ (hr)}^{-1}$$

The time required for 95 percent removal of starch-COD can be obtained from Equation 17.

$$\log \frac{L_o}{L_t} = k_s (t - t_o)$$

$$\log \frac{100}{5} = 0.256 (t - t_o)$$

$$\therefore t - t_o = \frac{1.301}{0.256} = 5.1 \text{ hours}$$

8. CONCLUSIONS

1. The removal of starch in an acclimated activated sludge system follows simple logarithmic kinetics for initial periods. However, in some cases after the initial period there is a divergence from the logarithmic relationship. The starch removal rate constant is dependent on food-to-microorganism ratio (F/M) and not on the initial solids.

2. The removal of total-COD, which includes starch degradation products, has a linear relationship with time as opposed to the logarithmic relationship of starch removal. In some cases there is a brief lag in the starch removal rate which is attributed to quantitative shock loading of the systems. The rate constant of total-COD removal is dependent on the initial mixed liquor suspended solids (MLSS) and not on F/M.

3. The effect of temperature on the removal rate of starch- and total-COD is quite significant. The energy of activation, ΔE , is of the order of 16,500 and 20,400 cal per mole for starch-COD and total-COD removal respectively.

4. In the aerobic systems there is no accumulation of starch breakdown products other than carbohydrates in the medium indicating that the further breakdown of these small carbohydrates is inside the cell.

5. Anaerobiosis of the acclimated activated sludge has no effect on starch degradation and initial adsorption of starch on the sludge, as long as the contents are well mixed. The rate of removal of starch for anaerobic conditions is similar to that for aerobic conditions. The total-COD and total-carbohydrate removal is very much retarded, and there is hardly any growth in the anaerobic systems.

6. Acclimation of the sludge is extremely important for high starch removal rates. The enzyme system responsible for starch degradation

(amylase) is constitutive in the case of glucose, maltose and Metrecal systems, and is adaptive in the case of bovine serum albumin system.

7. The large proportion of enzymes responsible for starch degradation in the systems tested are located on the cell walls with an insignificant amount liberated in the medium, especially during the log-growth phase. There is no perceptible inhibition of starch degrading enzyme systems by high concentration of substrate. Use of phosphate buffer in the systems tested allows less liberation of the starch degrading enzyme systems in the medium from the cell wall when compared to the systems with acetate buffer of the same pH. There is an indication of an intracellular starch degrading enzyme in the acclimated sludge which was not characterized.

8. The amount of starch substrate utilized for the synthesis of sludge varied from 58 to 64 percent.

9. Adsorption of starch by sludge is evident in all the systems tested but there is no good correlation between the amounts adsorbed per unit weight of sludge and the concentration of starch added or with the temperature. The amount of starch adsorbed on sludges varies from a high of 24 percent for acclimated systems to a low of 1.5 percent for non-acclimated systems. The amount of starch adsorbed is dependent upon the initial sludge concentration, temperature and acclimation characteristic of the sludge, but independent of the presence of oxygen.

10. Mechanism of starch removal in activated sludge system is the initial adsorption of starch on cells, followed by its degradation by the cell-wall-situated enzyme systems. The degradation products are liberated in the medium and subsequently assimilated by the cell. Thus, the mechanism of removal of colloidal substrates in activated sludge unit differs significantly when compared to simple small molecule substrates.

9. SUGGESTIONS FOR FUTURE WORK

The complexity of the removal of colloidal waste in an activated sludge system has already been emphasized. Only a small segment concerning the basic aspects of this problem has been investigated in this study. The investigation can be pursued further in two ways.

One line of approach would be to probe further into some of the aspects of the problem dealt with here. These may involve studying the removal of starch in the activated sludge system under different operating conditions. The operating variables that could be studied are effect of different pH, effect of nutrient deficiency and effect of the presence of other substrates in conjunction with starch. The location of the starch degrading enzymes on the cell walls can be further tested by degradation of the cell walls by lysozyme under conditions where the protoplasts are protected from disintegration and the cytoplasmic membranes are intact. Extraction of the enzyme from cell wall by lysozyme would undoubtedly pinpoint its location. The effect of starch adsorption on acclimation of sludge can also be studied in more detail, and reasons for the decreased adsorption after some days of acclimation may be investigated.

The use of contact stabilization process for the treatment of starch wastes may also be investigated in light of the findings of the present and future research.

Alternatively, the kinetics and mechanism of removal of colloidal substrates other than starch could be investigated. The substrates which could be studied are carbohydrates (glycogen, cellulose and pectin), proteins (albumins and casein) and lipids (lecithin and cephalin). The study of these different colloidal substrates in an activated sludge system would give a wider perspective to the results.

Further, it would be worthwhile to investigate the removal characteristics of industrial wastes having a large undefined colloidal content. Would the results of such an experimentation have any correlation with those obtained with pure colloidal substrates? The information thus obtained would be of great practical value.

The use of continuously operated activated sludge systems instead of batch systems used in the present study would be more profitable, since it may approach the actual situation in the field more closely.

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11. APPENDIX A

DETAILS OF ANALYTICAL PROCEDURE

Chemical Oxygen Demand Test (62)

Reagents

- i. Standard potassium dichromate solution, 0.25 N
- ii. Standard ferrous ammonium sulphate solution, 0.05 N
- iii. Sulfuric acid reagent

This was prepared by adding 22 grams of silver sulfate crystals to a nine-pound bottle of concentrated sulfuric acid

- iv. Ferroin indicator solution
- v. Mercuric sulfate crystals

Procedure

A 10-ml sample or an aliquot diluted to 10 ml with distilled water was mixed with 5 ml of standard potassium dichromate and 15 ml of sulfuric acid reagent. One gram of mercuric sulfate was then added. The mixture was refluxed for two hours with boiling chips in an Erlenmeyer flask. The flask was cooled, and 40 ml of distilled water was added. The excess dichromate in the mixture was titrated with standard ferrous ammonium sulfate using the ferroin indicator.

A blank consisting of 10 ml of distilled water instead of the sample, together with the reagents, was refluxed in the same manner. The calculations were done in the same manner as mentioned in the reference (61).

Starch-Iodine Test (66)

Reagents

i. Potassium iodide-iodine reagent

This was prepared by dissolving 2 grams of solid iodine in 20 ml of a solution containing 20 grams of potassium iodide, and then diluting to 1 liter with distilled water.

Procedure

Samples containing up to 2 mg/ml of starch were added to a 100-ml volumetric flask containing about 80 ml of distilled water. One ml of potassium iodide-iodine reagent was added. The intensity of the blue color formed was determined in the Lumetron colorimeter (Photovolt Corporation, Model 401) at 650 millimicrons after 15 minutes of color development, against a distilled water reagent blank. A set of standards (containing 0.5, 1.0, 1.5 and 2.0 mg of starch) were also treated in the same manner as the samples, in order to obtain a standard curve for estimating the amount of starch present in the samples. Figure 61 represents a standard curve for the potato starch substrate used in this study.

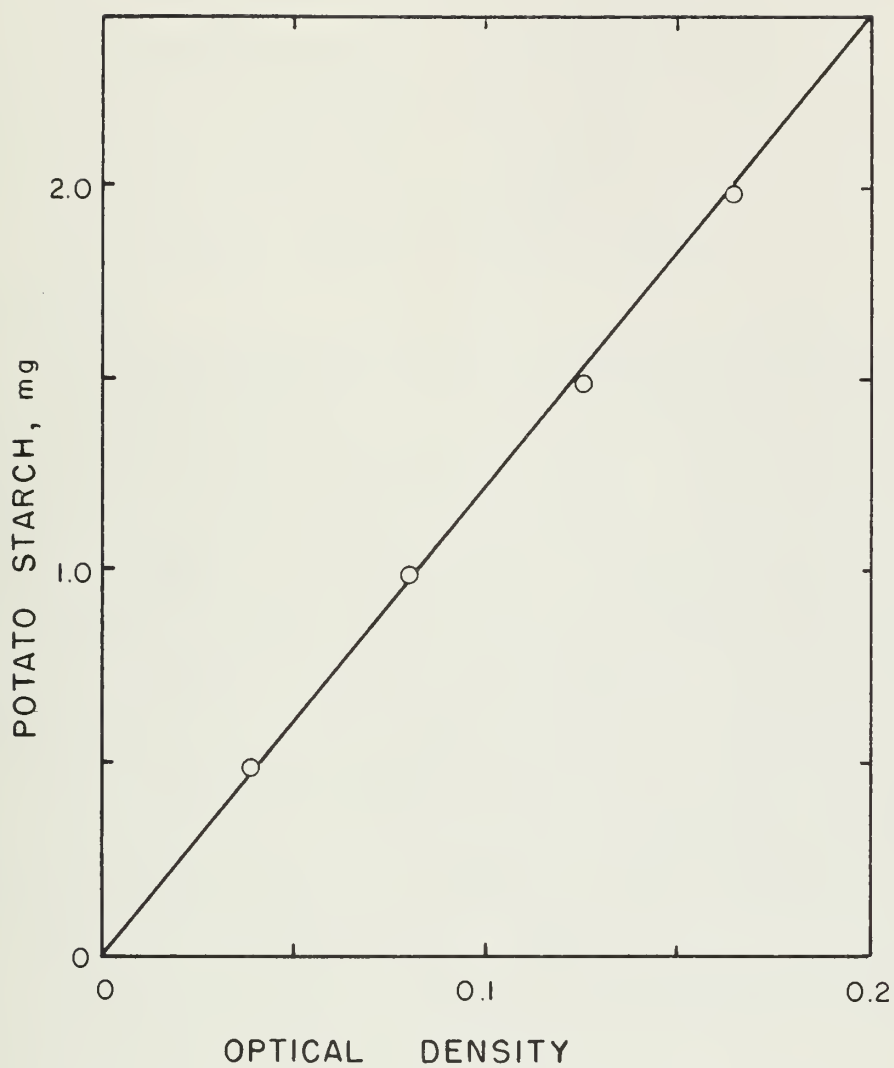


FIGURE 6I. STANDARD CURVE FOR POTATO STARCH- IODINE TEST

Total Carbohydrates-Anthrone Test

Reagents

i. Anthrone reagent

This reagent was prepared by dissolving 0.2 grams of anthrone in 100 ml of concentrated sulfuric acid. It was freshly prepared every time the test was performed and refrigerated for an hour prior to use, as the reagent is claimed to darken on aging.

Procedure

The test involved the use of a sample containing 10-100 micrograms of carbohydrate per ml. The sample was placed in a chilled test tube and made up to 5 ml with distilled water. Ten ml of freshly prepared anthrone reagent were quickly added and the reactants mixed by blowing air through the mixture. The tubes were covered with aluminum caps and placed in a boiling water bath for 10 minutes. After cooling the tubes, the optical density of the colored solution was determined at 620 mμ wave length in the Bausch and Lomb Spectronic 20 spectrophotometer against a reagent blank prepared with distilled water. A set of glucose standards was run each time the test was performed. Chilling of the reagents and the samples, prior to boiling, was practiced as recommended by several investigators (94)(95).

Folin Ciocalteu Test (69)

Reagents

i. Folin A reagent

This solution contained 20 g of sodium carbonate and 4 g of sodium hydroxide in 1 liter of distilled water. The reagent was stable for 2 to 3 months, if it was not contaminated.

ii. Folin B reagent

The reagent was prepared by dissolving 100 mg of sodium tartrate and 50 mg of copper sulfate in 10 ml of distilled water. In this form, the reagent was stable for a week, but if the 2 constituents were not mixed, the individual solutions were stable for longer periods.

iii. Folin C reagent

One ml of Folin-phenol reagent (supplied by Fischer Scientific Co.) was added to 1.2 ml of distilled water. This reagent was freshly prepared each time the test was performed, as it was stable only for an hour.

iv. Folin mixture

This reagent contained 1 ml of Folin A solution added to 49 ml of Folin B solution.

Procedure

One ml of a sample, or an aliquot diluted to 1 ml, containing 50 to 150 micrograms of protein was mixed with 5 ml of Folin's mixture in a test tube. The contents of the tube were allowed to stand for 15 minutes at room temperature. 0.5 ml of Folin C reagent was added to the tube and the contents mixed quickly in a vortex mixer. The mixture was allowed to

stand at room temperature for 30 minutes. If any organic debris was present, it was removed at this stage by centrifugation. The intensity of the color developed was measured in the Bausch and Lomb Spectronic 20 spectrophotometer at 700 m μ wave length against a reagent blank prepared with distilled water. A set of standards prepared with bovine serum albumin fraction V was assayed every time in order to obtain a standard curve.

12. APPENDIX B

ACCLIMATION OF UNITS

Determination of Solids Balance

The seed sludge (500 mg) from the continuous Metrecal fed activated sludge unit was used to start the acclimation units for each of the substrates used. The concentration of substrate fed every day was about 1500 mg/l in the case of starch, maltose and glucose units (1000 mg/l in the case of bovine serum albumin unit). A third of the mixed liquor was wasted every day. The mixed liquor suspended solids, representing the biologically active mass, was determined from time to time. The steady state conditions were attained when the amount of synthesis of sludge by utilization of substrate was balanced by the amount of sludge wasted every day. Figure 62 indicates the attainment of acclimation of Metrecal sludge to starch substrate over a period of time. The steady state MLSS concentration for starch substrate was 1330 mg/l under the conditions of operation.

Figures 64, 65 and 66 represent the attainment of steady state condition MLSS in the glucose, maltose and bovine serum albumin substrate systems respectively starting with Metrecal sludge.

In the case of starch, acclimation was also determined by the attainment of optimum substrate removal kinetics as shown in Figure 63. It was evident it took only about 5 days for the Metrecal sludge to get acclimated to starch substrate.

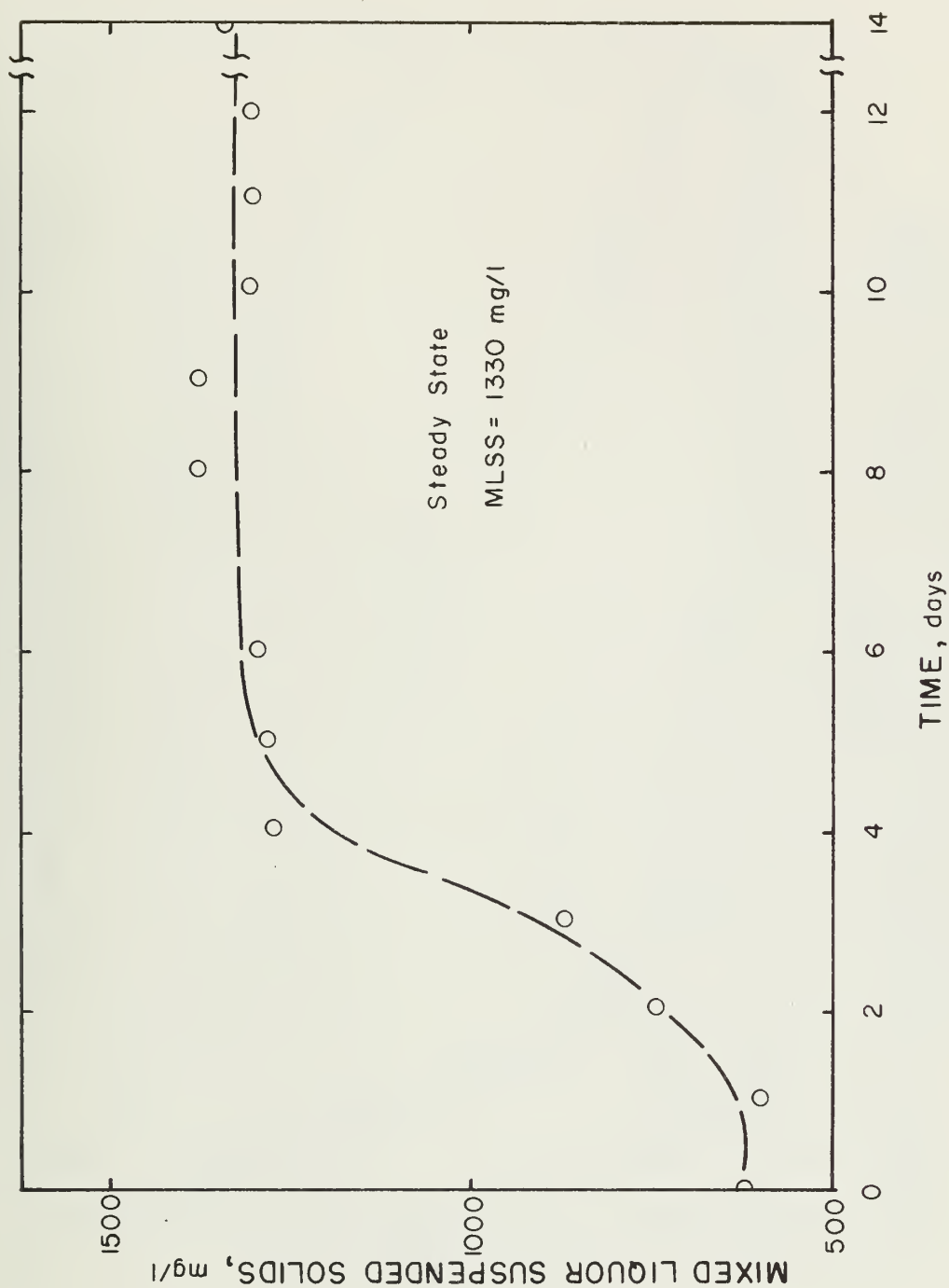


FIGURE 62. ACCLIMATION OF METRECAL SLUDGE TO POTATO STARCH
SUBSTRATE - MLSS BALANCE

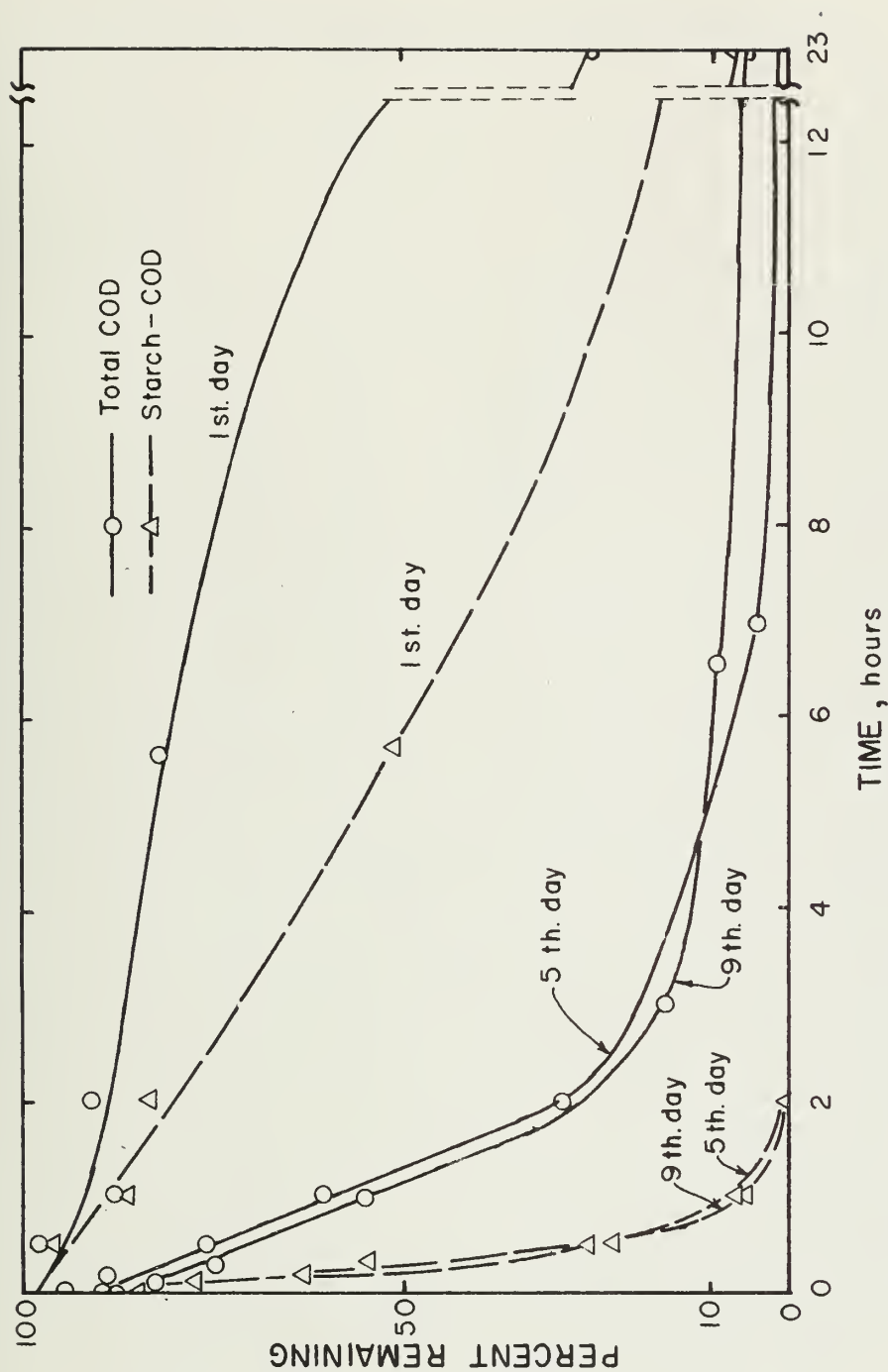


FIGURE 63. ACCLIMATION OF METRECAL SLUDGE TO POTATO
STARCH SUBSTRATE—CHANGE IN SUBSTRATE
REMOVAL RATES

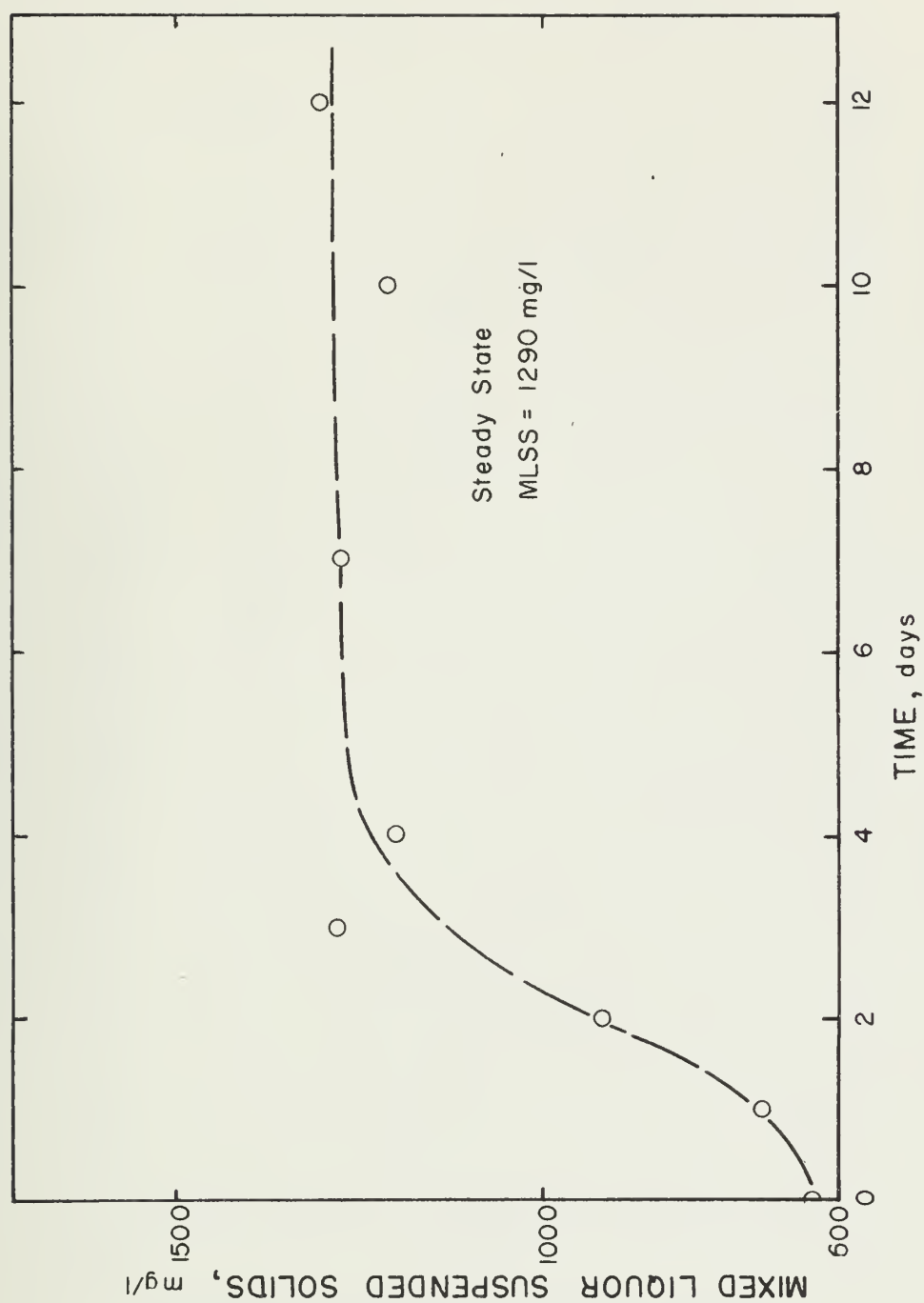


FIGURE 64. ACCLIMATION OF METRECAL SLUDGE TO GLUCOSE
SUBSTRATE - MLSS BALANCE

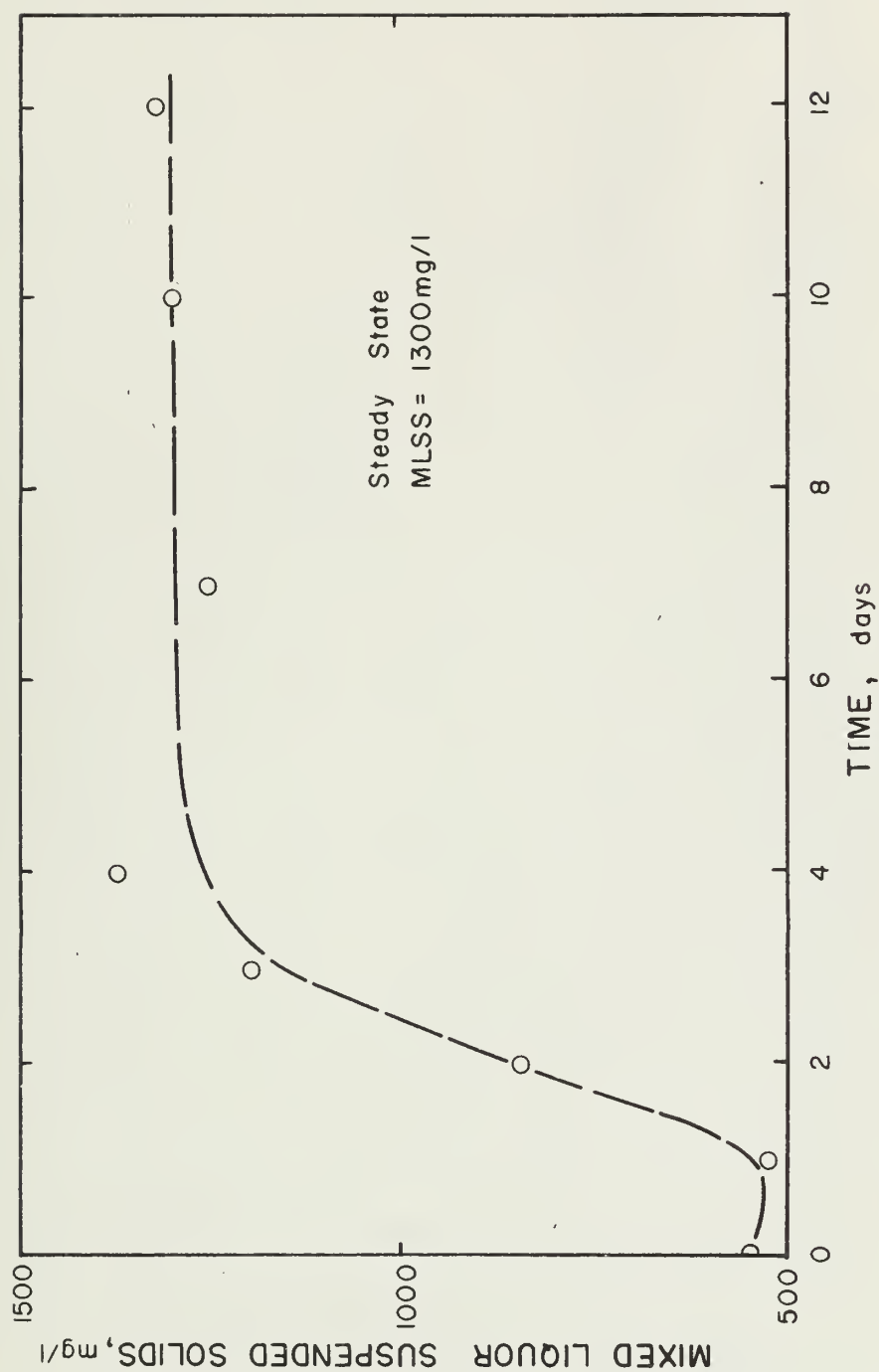


FIGURE 65. ACCLIMATION OF METRECAL SLUDGE TO MALTOSE
SUBSTRATE - MLSS BALANCE

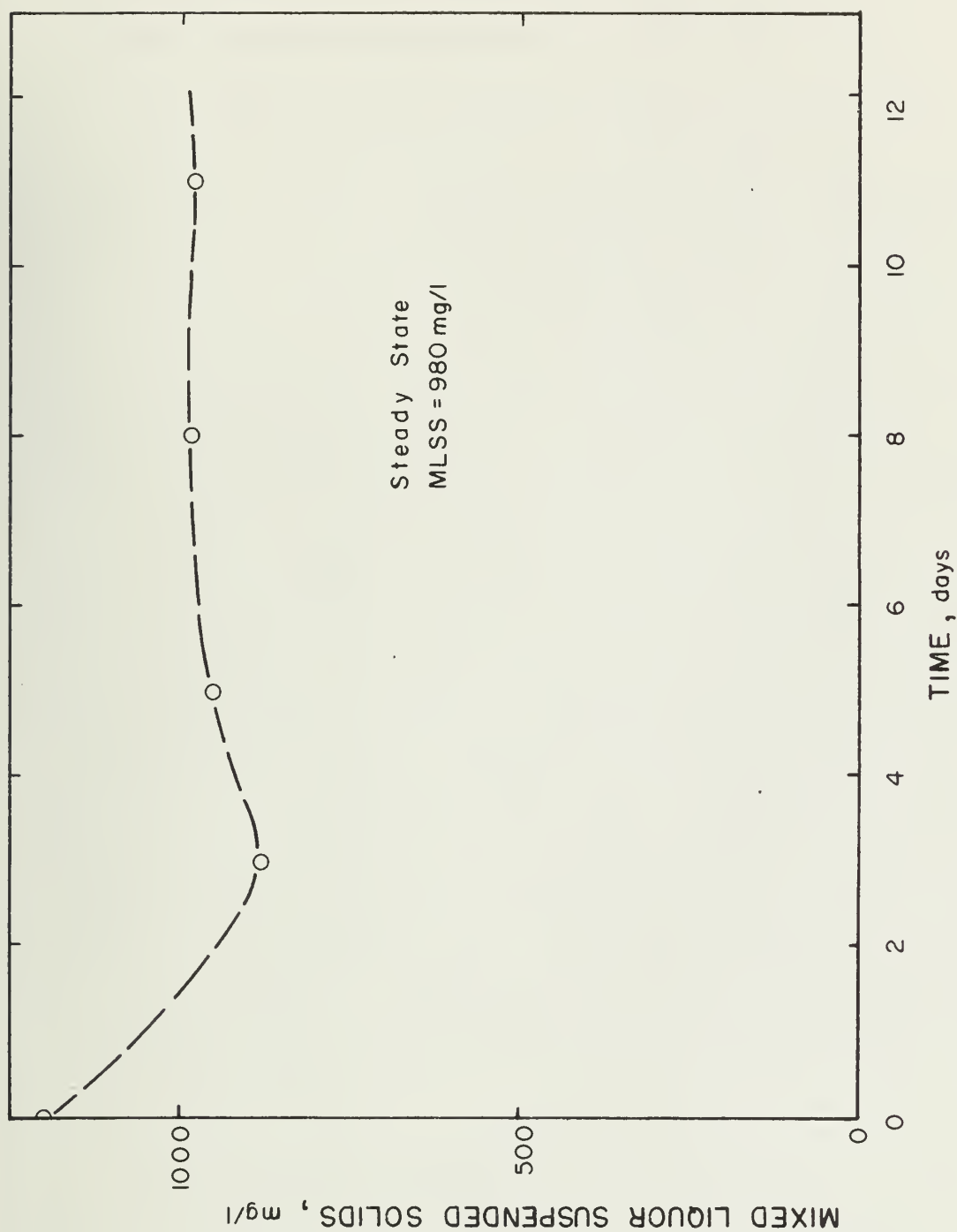


FIGURE 66. ACCLIMATION OF METRECAL SLUDGE TO BOVINE
SERUM ALBUMIN SUBSTRATE-MLSS BALANCE

13. VITA

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After finishing his pre-college education at Colvin Collegiate School in Lucknow, he qualified to study at Bengal Engineering College, Sibpur, Howrah (affiliated to the University of Calcutta, India) in 1953. During the entire period of study at Bengal Engineering College, he was awarded a merit Scholarship for high scholastic grades. In 1957, he received the Bachelor of Engineering degree in Civil Engineering from the University of Calcutta, graduating with a First Class. From 1957 to 1960, he was engaged in civil engineering practice, primarily design and construction of water supply and sewerage facilities for the Durgapur Steel Project Township.

In 1960, he undertook graduate work at the University of Illinois.

He was awarded the degree of Master of Science in Sanitary Engineering. The title of his Master's thesis was "Effect of Biological Slime on the Retention of Alkyl Benzene Sulfonates on Granular Media." Since 1960, he has held a Research Assistantship. Initially, he was associated with a research project, "Fate of Synthetic Detergents in Soils and Ground Waters," which was supported by the United States Public Health Service, and later he was associated with the research project, "Initial Rapid Removal of Organic Matter by Microorganisms in Treatment of Waste Waters," also supported by the United States Public Health Service. He was elected a member of Sigma Xi.

The author has co-published the following papers:

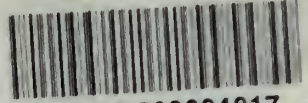
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